ECOLOGY AND THE EVOLUTION OF ANTI-PREDATORY MORPHOLOGY WITHIN BUSYCONINE WHELKS: A PLIOCENE TO RECENT PERSPECTIVE

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ABSTRACT

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My dissertation focuses on the ecological circumstances associated with the evolution of heavily ornamented morphologies within Busyconine whelks. Busyconine whelks display a variety of shell forms in the Recent and fossil record, with heavily ornamented shells concentrated in the southern part of the modern species *Busycon carica*'s range. In the fossil record, heavily ornamented shells recur in temporally disjunct populations over variable geographic extents from the Pliocene onwards. I investigated the function of shell sculpture (such as the tumid ridge, increased shell thickness, and spines), as well as fitness costs (such as reduced growth) associated with producing and maintaining heavily ornamented shells. I used stable isotopes to construct growth curves, and compared growth estimates to prior studies throughout *B. carica*'s range.

In modern populations of *B. carica*, increased ornamentation confers a significant benefit when dealing with shell crushing predators. Growth curves constructed from stable isotopes show a latitudinal trend in growth rates, with slower growth in northern populations, and also revealed diminished growth in the south within populations for heavily ornamented individuals, as compared to weakly ornamented individuals. Some prior growth studies have ignored episodicity of growth in *Busycon*; this has led to inaccurate estimation of rates. Future work should focus on both incorporating intervals of non-growth into models derived from mark-recapture studies, and also on further examining growth costs associated with the development of

heavily ornamented morphologies. This work also highlights the importance of standardizing for growth rates, body size, and degree of ornamentation when calculating repair frequencies.

In the fossil record, adaptations are best developed in the Pliocene, and diminish sharply in abundance across the Plio-Pleistocene boundary coeval with a regional extinction event. The use of an ecometric approach, which advocates using the presence or absence of traits to assess environmental and ecological conditions, suggests that the maintenance of adaptations following the extinction event was diminished, likely as a combined response to both deteriorating environmental conditions and lower predation rates. Higher predation rates and more permissive environmental conditions in the late Pleistocene and Recent are correlated with increased abundance of defensive features.

BIOGRAPHICAL SKETCH

Mary was born in Buffalo, New York in 1984 and grew up in Tonawanda, New York, where she was privileged to have access at a young age to the abundant Devonian fossil fauna of the region. Her father, an avid fisherman, took Mary and her sisters Carol and Sarah on many fossil expeditions in Eighteen Mile Creek, where he divided his time between catching bass and helping the girls identify their fossils. At Eighteen Mile Creek, Mary learned to recognize both concretions (including western New York's "turtle rocks") and the brachiopod *Mucrospirifer mucronatus*, and spent many fruitless hours scanning the gorge walls in hopes of discovering the area's first *Tyrannosaurus rex* skeleton. These trips fueled her childhood ambition of becoming a paleontologist.

Mary initially started college at SUNY Geneseo in 2002 with the intention of pursuing a degree in Elementary Education with a French minor. After taking a course on Historical Geology during her first semester with Dr. D. Jeffrey Over, she became increasingly interested in Paleontology and Geology. Over the next few semesters, Mary took several more courses in Geology, and then switched to a Geology major at the end of her sophomore year. At Geneseo, she completed a senior Honors Thesis under Dr. Over's advisement on a conglomeratic lag deposit in a local sandstone complex.

In 2006, Mary began her pursuit of a PhD at Cornell University in Ithaca, New York.

DEDICATION

My dissertation is dedicated to my parents, Janet and Victor Kosloski, who have supported me in every sense of the word for the past 27 years. Thank you for telling me always that I could be anything I wanted, thank you for sharing your love of nature, thank you for taking me camping, and thank you for being not just my parents, but also my friends. You two are the very best.

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At Cornell, I have been supported by a McMullen Fellowship, numerous TA appointments through the Department of Earth and Atmospheric Sciences, research assistantships through the Paleontological Research Institution, and through the Engineering Learning Initiatives program as a TA trainer and as the TA Fellow. Conference travel while at Cornell has been generously supported by the Department of Earth and Atmospheric Sciences Kaufmann Grant, the Paleontological Research Institution, and the Cornell Graduate School.

I have also received support in my research from the University of Georgia's Marine Extension Service, particularly Alan Power, Randal Walker, and Brian Corley. Brian took me out in the salt marshes to hunt for whelks on several occasions, and other members of the team provided both shells and data that I could not have completed my dissertation without, while additionally providing a long record of outstanding research on whelks.

Jerry Harasewych at the Smithsonian National Museum of Natural History, Lauck Ward at the Virginia Museum of Natural History, Leslie Skibinski at the Delaware Museum of Natural

History, and Karen Walker and Irvy Quitmyer at the Florida Museum of Natural History all provided access to collections throughout my dissertation.

The program PAST is freely available, and has been a great help in various statistical analyses carried out throughout this body of work.

I have been incredibly fortunate in my mentors, educators, and friends over the past 27 years, and could not have gotten through the past six years without the tools and support that they have given me.

In middle school, Grace Lawrence was both an excellent teacher, and a memorable example of a female naturalist. At Kenmore East High School, Ralph Critelli sparked my interest in the Earth Sciences by both creating engaging lectures, and by organizing a hundred-student plus earth-science and white water rafting weekend. I suspect strongly that his efforts at making science interesting, exciting, and fun are largely if not almost solely responsible for Kenmore East High School's high output of geologists.

At Geneseo, during Freshmen Orientation, William Edgar and his wife, Stacey Edgar, recommended that I take a course in Earth Sciences with D. Jeffrey Over to fulfill my sciences requirement. This course re-introduced me to Geology and Paleontology after a long gap, and I am so thankful that the Edgars pointed me towards it. Jeff has served as a wonderful mentor and advisor, both during my initial four years at Geneseo as an undergraduate as well as this fall, when I had the opportunity to return and teach as an adjunct. He has also demonstrated just how

good a very well delivered lecture could be, and has been an excellent example of I aspire to be as a professor. Scott Giorgis, Dori Farthing, Amy Sheldon, Richard Hatheway, and Richard Young also provided really wonderful support and mentoring throughout my time at Geneseo.

At Cornell, I am hugely indebted to Snee's very excellent staff. Of particular import, Steven Gallow has more than once saved me from technological disasters of epic proportions, and Savannah has provided the logistic and emotional support necessary to get through a dissertation. Linda Tompkins and Teresa Jordan have also both been a constant source of encouragement and kind advice.

My committee members, John Cisne and Catherine Drew Harvell, have helped me to think outside of the somewhat narrow scope of a dissertation, while providing feedback that improved hugely the quality of this manuscript. Greg Dietl introduced me to both *Busycon* and field work in the Coastal Plains, and has served as an incredible example of a scientist as well as a really wonderful mentor. Warren Allmon has provided energy and encouragement, and has always looked out for my best interests even when I had no idea what they were. He has also supported my pursuit of all sorts of teaching opportunities and experiences, and I could not have lucked into a better role model or a more supportive advisor for going into academia. I have been privileged to work with all of you, and have benefited greatly.

I have also been privileged to be a part of the Snee student community, and am particularly grateful for the friendships of Tiffany Tchakirides, Greg Kirkpatrick, Steve Holtkamp, Sander Hunter, Gregg McElwee, Brita Lorentzen, and Veronica Prush. Steve

Durham and Dana Friend have very kindly tolerated me as an office mate during the past year as I've written up, and have been very fun to work with. I'm excited that they will be carrying on the Paleontology tradition at Cornell—you two are great. Outside of the department, Sandy LaBuda and Sarah and Jake Clements have been some of my very best friends for a decade and more, and have been everything from great listeners to really fun breaks from work to second homes. I hope we remain friends for all of the rest of the decades, too.

Ursula Smith, my paleo-sibling, cannot be thanked enough. Ursula has been a Thai food date, a road trip buddy, briefly a house mate, a brilliant example of a researcher, an excellent confidant, and an introducer to many of the finer things in life (such as Australian chocolates, Coco Chanel Mademoiselle, and crocheted nautiloids). She has advised me on everything from geometric morphometrics to research grants to conference wardrobes (by her intervention alone, I have been saved from wearing black shoes with brown skirts). Australia is lucky to have her.

Bill Barnhart has been both a best friend and partner, and has kept me fed, sane, happy, and working hard for the past year. He has been incredible, and he has made me stronger (emotionally, logistically, directionally, and grammatically, as well as in terms of work ethic and cooking repertoire). I'm looking forward to more bacon, walks, and DMB concerts in the future—it has been a good, good time.

Lastly, my family has been a great and constant blessing. My sisters, Carol and Sarah, continue to serve as inspirations for the kindness and goodness that they work constantly to bring to the people (and animals) around them. They have served as examples of how to go after the

things that matter. The discussions we have had have shaped an enormous part of who I am—certainly the better part, in both senses of the word. They are incredible friends, and I'm glad we'll all finally be on the same continent next year. My parents, Janet and Victor Kosloski, have been the very best parents I could have ever hoped for. Thank you so much for showing me the world around me, for giving me every opportunity to succeed, for always standing with me through all of my decisions, efforts, and pursuits, and for being the kind of people I'd like to be someday myself. All of this has been very fun, and I'm glad I've had you all with me through it.

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CHAPTER 1

REPAIR SCAR STANDARDIZATION 101: WHY WE NEED TO STANDARDIZE OUR DATA

ABSTRACT

The use of repair scar frequencies is increasingly common in both ecological and paleontological literature. The standardization techniques employed, however, are variable between studies, with some studies standardizing extensively and some incorporating unstandardized data. I examined the effects of different types of data standardization (e.g., for body size, shape, and exposure time) on repair frequency for a dummy data set of 1,000 specimens of an imaginary marine gastropod species that varies across its geographic range in morphology and life history. I based assumptions for this hypothetical system on literature reports of living marine gastropod species. Results highlight the importance of careful standardization of samples at the lineage level, as well as by body size, shape, and exposure time, as well as the utility of conversion of repair frequencies (percent of shells with at least one repair scar) to rates of repair scar accumulation (number of scars per unit of time). The use of these standardization techniques in future studies should both enhance our ability to detect ecological signals, and facilitate easier comparisons between studies and study systems.

INTRODUCTION

Over the past several decades, studies of shell-crushing predation have become increasingly common in the paleontological literature (e.g., Vermeij, 1977; Schoener, 1979; Vermeij et al., 1980; Vermeij et al., 1981; Vermeij, 1987, 1993; Allmon et al., 1990; Cadée et al., 1997; Dietl & Alexander, 1998; Kowalewski, 2002; Leighton, 2002; Zuschin et al., 2003; Alexander & Dietl, 2003; Cintra-Buenrostro & Flessa, 2005; Huntley & Kowalewski, 2007; Moody & Aronson, 2007; Cadée, 2011; Stafford & Leighton, 2011). Because unequivocal evidence of lethal predation is often difficult to recognize (though see Oji et al., 2003; Cintra-Buenrostro, 2007; Zaton & Salamon, 2008; Kosloski, 2011 for examples), what we know about the history of shell-crushing predation largely comes from the fossil record of sublethal predation preserved as repair scars. When prey organisms are sublethally damaged, they will often repair their shells, leaving behind a record of diagnostic traces on the shell (Figure 1.1) (Vermeij et al., 1981; Alexander & Dietl, 2003).

Figure 1.1. Repaired shell of Busyconine whelk. Scale bar=1 cm.

Repair frequency (RF) can be expressed as either the percentage of individuals possessing at least one repair ("percent method"), or as the total number of repairs divided by the number of individuals in a sample ("scars-per-shell method") (Alexander & Dietl, 2003). Calculating RF in this way may give a more accurate assessment of the selective pressure imposed on the population as a whole, as opposed to the scars per shell method, as the former calculation accounts for the percentage of the population actually experiencing selection (see Alexander & Dietl, 2003; Kowalewski et al., 1997). Changes in RF, expressed by either method, can be challenging to interpret, however; increases may represent either more frequent predatory attacks, or decreases in predator success, either from diminished predator strength or increased prey morphological defenses (Vermeij, 1982, 1987, 2002; Schmidt, 1989; Leighton, 2001; Alexander & Dietl, 2003; Zuschin et al., 2003). While viewing RF as an index of selection strength (as advocated by Vermeij, 1982) bypasses some of these issues, trends in RF have occasionally emerged in the literature that are counterintuitive, given knowledge of predator abundance, or presumed predation pressure. For example, De Wolf et al. (1998) examined variation in macrostructure and predation pressure in periwinkles (*Littorina striata*) from Macaronesia, and found higher repair frequencies in northern populations (in contrast to their predictions of more intense predation to the south). De Wolf et al., however, did not account for inter- and intra-populational differences in growth rate or the effects of enhanced shell sculpture on the accumulation of repair scars. In this case, counter-intuitive trends may not have been an issue of ecological interactions leaving undetectable or misleading traces, but of incomplete consideration of variables that impact RF.

Additional standardization techniques may increase the usability and comparability of information contained in the trace record by accounting for factors known to modify RF. As the

literature on predator/prey dynamics has expanded, it has become increasingly apparent that a variety of different traits, ecological characteristics, and levels at which data are sorted may affect both the apparent frequency of predation, and its likelihood of leaving identifiable traces on prey organisms (Table 1.1). My goal in this thought exercise is to illustrate some common design flaws in recent studies (in effect, an explanation of why we need to standardize), and to lay out a set of recommended standards ("recipes for reality") that could be adopted to increase both the ecological relevance and general utility of RF data, and that address many of the issues mentioned in the table below. The methods outlined below, which are necessarily illustrative instead of comprehensive, will facilitate comparisons between studies, and produce more ecologically relevant and reliable information.

Factor	**Selected References**
Prey body size/size structure	Vermeij, 1982; Schmidt, 1989; West et al., 1991; Cadée et al., 1997; Kowalewski, 2002; Leighton, 2002
Prey growth rate	Cadée et al., 1997; Alexander & Dietl, 2003; Dietl & Alexander, 2009
Prey shell microstructure	Vermeij, 1993; Alexander & Dietl, 2003; Zuschin et al., 2003
Prey shell macrosculpture/shape	Vermeij, 1982; Schindel et al., 1982; Schmidt, 1989; West et al., 1991; Vermeij, 1993; De Wolf et al., 1998; Walker, 2001; Zuschin et al., 2003; Lindstrom & Peel, 2010
Habitat	Cadée et al., 1997; Zuschin et al., 2003
Predatory regime (predator abundance, intensity, etc.)	Vermeij, 1982; Vermeij, 2002; Moody & Aronson, 2007
Prey life habit	Vermeij, 2002
Prey adaptive syndrome	Vermeij, 1987, 1993, 2002; Dietl & Vermeij, 2006
Sample size	Kosloski et al., 2008
Taxonomic sorting of data set	Schindel et al., 1982; Dietl & Alexander, 1998; Alexander & Dietl, 2001; Vermeij, 2002; Alexander & Dietl, 2003

Table 1.1. Factors that might explain variation in RF data, and that might therefore be targets for standardization. References listed are examples of studies which have attributed variation to the respective factor.

Why standardize?

The Merriam-Webster dictionary (merriam-webster.com, accessed 5/9/2012) defines the act of standardizing as either "compar(ing) with a standard", or "bring(ing) into conformity with a standard", with a standard defined as "something established by…general consent as a model, or example". As repair scar analyses have become increasingly utilized, several authors have expressed concern that the use of unstandardized data may obscure trends (e.g. Alexander & Dietl, 2001; Vermeij, 2002; Alexander & Dietl, 2003). Recent analyses encompass a hierarchy of possible standardization techniques, ranging from the use of completely unstandardized, assemblage-level data sets, to data sets that are at least somewhat constrained taxonomically (e.g., at the genus or species level). Taxonomically constrained data sets may be further standardized by body size (binned by size classes), or, in rare cases, standardized by age (see Cadée et al., 1997; Alexander & Dietl, 2003; Dietl & Alexander, 2009, for discussion of effects of ontogenetic changes in growth rate on repair frequency).

To determine the extent to which different levels of standardization can affect RF (and to illustrate how critical standardization may be), I use a dummy data set (following Leighton 2002) composed of observations on the imaginary marine gastropod, *Conchus imaginarius*, by a variety of metrics. The ultimate goal of these progressive standardization techniques is to make sure that comparisons are between samples with equivalent likelihoods of accumulating repair scars. Employing these techniques will increase the chances that studies will capture ecologically relevant signals. I also discuss problems with assemblage level data, as well as sample size issues. My data are initially standardized taxonomically, and sample sizes are large (500 individuals per sample). Additionally, I standardize by body size (shell length, in 50 mm size classes), exposure time, and shape (Table 1.1). In this chapter, I use the term "shape" as per

Bookstein et al. (1985; p. 259), wherein "shape" is referred to as "the geometry of the organism after information about scale, position, and orientation has been removed". Thus, shape standardization accounts for differential development of, for example, anti-predatory defenses (such as shell thickness, spinosity and ornamentation), as well as differences in morphology resulting from local adaptation or phenotypic plasticity.

ASSEMBLAGE--LEVEL DATA

Assemblage-level data represent a special problem for analyses (as discussed below), and are generally rare in the literature. Many of the issues inherent in assemblage level data also represent larger magnitude versions of likely issues with lineage-level data, which is what the rest of the paper focuses on. For this reason, a discussion of assemblage level data may help illustrate issues that also manifest at the species level during analyses.

Assemblage-level data encompasses bulk samples or outcrop surveys from localities, which tend to incorporate a variety of higher taxa (Kowalewski, 2002). Samples may be further sorted by size (i.e., by sieving during processing), or at lower taxonomic levels (e.g., Kowalewski, 2002; Sawyer & Zuschin, 2011). Assemblage-level comparisons must consider a range of potential issues, however, because RF may be different among the species in the assemblage for many reasons (e.g. Schindel et al., 1982; Schmidt, 1989; Dietl & Alexander, 1998; Alexander & Dietl, 2001; Alexander & Dietl, 2003). For instance, shell microstructural differences between taxa (e.g. cross-lamellar vs. nacreous structures; high vs. low organic content) can modify organism's capacities to sustain damage (see Alexander & Dietl, 2003; and Zuschin et al. 2003 for a review). For example, in Lake Tanganyika, many gastropod species

construct shells with multiple crossed lamellar layers, and the development of these features correlate with increased shell strength and resistance against predators (West & Cohen, 1996).

Macrostructural variation in shell sculpture also affects the likelihood of accumulating and recognizing repairs, further complicating large scale comparisons (e.g., Bertness & Cunningham, 1981; Schindel et al., 1982; Schmidt, 1989; West et al., 1991; De Wolf et al., 1998; Walker, 2001). For instance, Schmidt (1989) found highest repair frequencies among squat species with large apertures and low levels of shell ornamentation (e.g., *Tegula* and *Natica*) as opposed to higher spired species with narrower apertures and higher levels of shell ornamentation (e.g., *Cerithidea*, *Acanthina*) in modern shell assemblages from the northern Gulf of California. Similarly, Lindstrom & Peel (2010) found a correlation between shell shape and repairs for Jurassic vetigastropods, with high spired trochiform shells displaying the lowest repair frequencies.

Size is another characteristic that varies both between habitats, assemblages, and temporally (e.g., Rex et al., 1999; Roy & Martien, 2001). Predators may choose to handle prey items in a particular size range, and/or only be successful attacking prey below a certain size (e.g. Paine, 1976; Elner & Hughes, 1978; Vermeij, 1982; Boulding, 1984; Creswell & McLay, 1990; Juanes & Hartwick, 1990; West et al., 1991; Yamada & Boulding, 1998; Leighton, 2002), and changes in body size between samples should be assessed. Thus, variations in the morphology (both shape and size) of constituent species between assemblages (due to either changes in species composition, or evolutionary change within the lineages present) are likely to modify RF, even if predation intensity is equivalent. Assemblage-level data also may include mixtures of habitat types. Structural complexity (e.g., variation in seagrass cover, oyster reefs vs. sand flats) may affect the ability of predators to effectively forage for prey, and may also

mediate interference interactions between different predators (e.g. Peterson, 1982; Hughes & Grabowski, 2006). Predator preference for specific habitat types also plays an important role in between-habitat differences in predation intensity. Micheli and Peterson (1999) found that the intensity of blue crab (*Callinectes sapidus*) predation on hard clams (*Mercenaria mercenaria*) occurring on oyster reefs in North Carolina was dependent on the surrounding habitat, as blue crabs were less likely to cross open sand bottoms (as compared to vegetated areas) to reach oyster reefs. Blue crabs have also been found to both prefer and forage most effectively in mud and sand/mud habitats (Arnold, 1984). Percentage seagrass cover significantly affects predation and siphon nipping on infaunal bivalves (Irelandi, 1994). Given between-habitat variation in both community structure and predation, comparisons at the assemblage level should be carefully controlled (i.e., standardized) to make sure that habitats remain consistent and do not represent mixtures of disparate habitat types; otherwise, differences in organismal behavior will likely affect predation intensity and consequently, RF.

Even if similar habitats are compared, spatially and temporally distinct assemblages are often characterized by different suites of organisms, with different "adaptive syndromes" (Vermeij, 1987, 1993, 2002; Dietl & Vermeij, 2006) resulting from local adaptation as well as other evolutionary processes, such as genetic drift (e.g. Vermeij, 1987; Cadée et al., 1997; Alexander & Dietl, 2001; Kowalewski, 2002; Leighton, 2002; Vermeij, 2002). Geographic differences in predatory regimes (i.e., predation intensity, predator abundance, and/or effectiveness), for instance, have been implicated in the evolution of clinal variation in prey species' behavior, diversity, and morphology (e.g., Stachowicz & Hay, 2000; Sanford et al., 2003; Laurila et al., 2008; Schemske et al., 2009; Freestone et al., 2011; Kosloski, 2012), and temporal variation in these factors is an overarching theme in paleo-ecological literature (e.g.,

Vermeij, 1977, 1987; Kelley & Hansen, 1993; Dietl, 2003; Huntley & Kowalewski, 2007). Changes in the composition of predatory guilds can potentially shift which species are "preferred" prey, as well as the relative efficacy of dominant predators vs. available prey species.

Differences in escape responses or other behavioral defenses of prey may further alter repair frequencies (even with equivalent predation intensity) at the level of individual species, and certainly between species. Vermeij (2002) discussed disparity in life habit, using theoretical assemblages of slow-burrowing bivalves vs. rapidly burying bivalves: differences in adaptive syndromes between the two taxa (where the first avoids predation by being well-armored, whereas the second avoids predation by limiting subjugation) would lead to higher apparent RF for the first taxa, relative to the rapid burrowers.

Many large-scale studies of predation (e.g., Huntley & Kowalewski, 2007, etc.) propose an increase in predation across the Phanerozoic, which is in line with Vermeij's hypothesis that biotic interactions have escalated through time (Vermeij, 1987, 1999): while I sympathize with these results, variation in the level at which these analyses are carried out (e.g., whole assemblage, global scale), as well as variation over time in habitat types, body sizes, lineages and taxa, and adaptive syndromes, makes their validity difficult to assess (see Dietl & Vermeij, 2006 for a discussion of complicating factors). Given the above conditions, assemblage-level data must be viewed as an amalgam of potentially heterogeneous signals, which may be extremely difficult to interpret. Many authors have suggested that comparisons will only be relevant among morphologically and functionally equivalent taxa (e.g., Vermeij 1987, Alexander & Dietl 2001, Vermeij 2002, Alexander & Dietl 2003). We therefore recommend that assemblage-level comparisons consider samples with similar taxonomic compositions, morphologies, habitat types, and adaptive syndromes.

STUDY SYSTEM

My fictional marine gastropod, *Conchus imaginarius*, ranges along the eastern (north-south trending) coast of the land mass Darwinia, where it encounters a range of abiotic environmental conditions and different durophagous predators—most importantly shell-crushing and peeling crabs, which peak in abundance to the south. *Conchus imaginarius* has two morphs, commonly recognized as subspecies but sometimes overlapping in geographic range (a not-uncommon occurrence in the real world; e.g., sympatric sister species, see Krug, 2011): *Conchus imaginarius imaginarius* and *Conchus imaginarius ornamentus*. The two morphs differ in both shell thickness (the latter subspecies has an average thickness of 5 mm, as compared to an average thickness of 2 mm for the former species) and spinosity: *C. i. ornamentus*, as its name suggests, has large spines protruding from the shoulder of its shell at 180 degree intervals (Figure 1.2). The *C. i. imaginarius* morph is a common member of the intertidal fauna throughout the entire species' range, whereas *C. i. ornamentus* is only common in populations to the south, where it frequently occurs in equal abundances to the other morph (Figure 1.2).

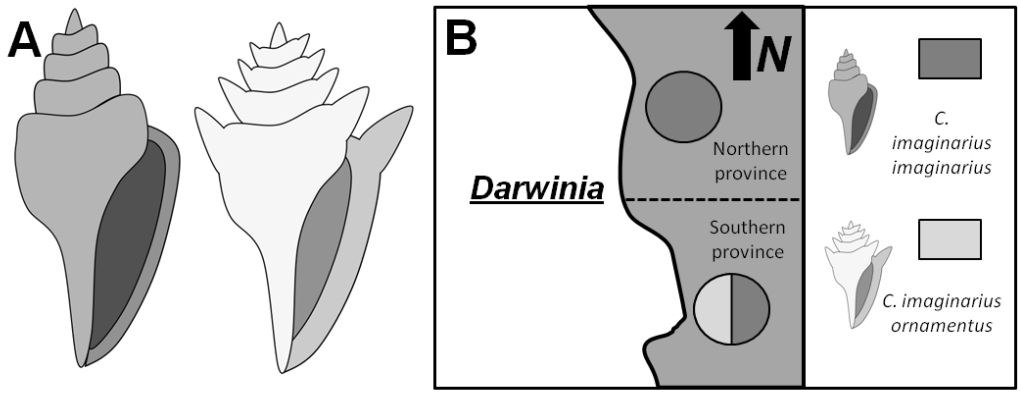

Figure 1.2. *Conchus imaginarius*, *C. imaginarius imaginarius* morphology on left; *C. imaginarius ornamentus* morphology on right (A). Map on right (B) shows land mass **Darwinia** and relative abundance of the two morphologies in northern and southern parts of range (pie diagrams).

C. imaginarius is an indeterminate grower (growth occurs until death, instead of stopping at a particular size or ontogenetic stage), with growth rates invariant and continuous throughout ontogeny: in my study, I consider individuals between 0 mm-200 mm shell length from the apex to the tip of the siphonal canal. Size distributions are slightly right-skewed to the south and left-skewed to the north (Figure 1.3). *Conchus imaginarius* grows much more rapidly in the southern part of its range, with growth rates on average 3 times as fast as observed in populations to the north.

RF in *C. imaginarius* is calculated as the number of individuals possessing at least one repair scar on the final whorl, divided by the total number of individuals in the sample (percentage method; e.g., Alexander & Dietl, 2003; Cadée et al., 1997). Laboratory predation experiments on *C. imaginarius*, standardized for the time prey are exposed to their predators, show a difference in the likelihood of accumulating repair scars between the two morphs: *C. i. ornamentus*, as a result of its increased spinosity and greater thickness, is five times more likely to survive a predatory attack than *C. i. imaginarius*. *C. i. ornamentus* is also less likely to suffer severe shell damage as a result of its increased morphological defenses, which result in an approximately two-fold increase in repair scar formation in smaller size classes.

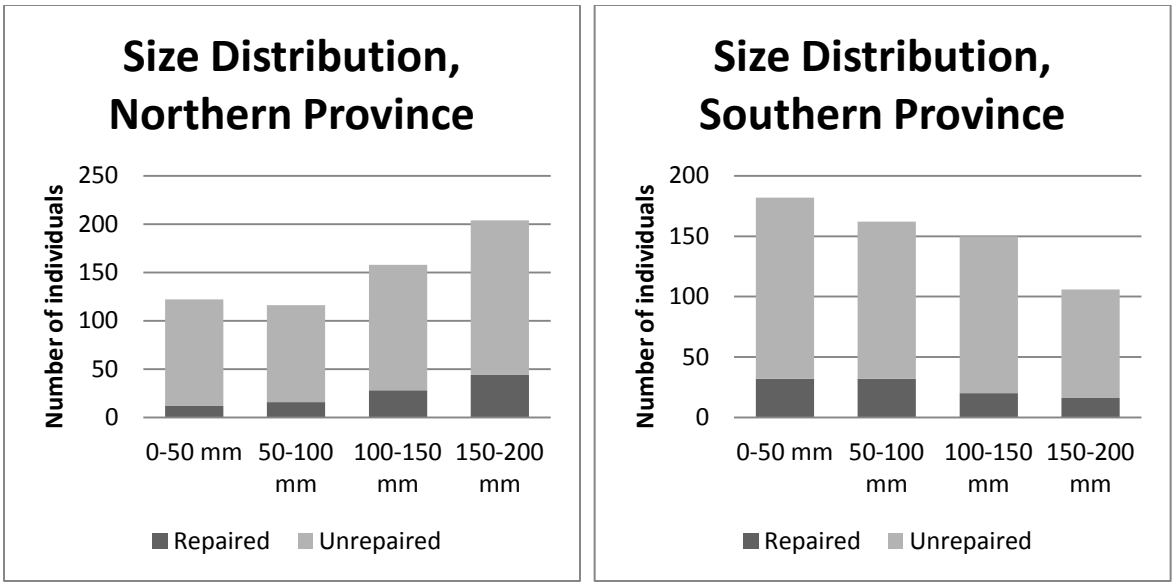

Figure 1.3. Length distributions and distribution of repaired individuals within the northern and southern provinces for northern and southern populations of *C. imaginarius.*

System Justification

While my imaginary system may at first seem perhaps unreasonably idealized for arguing that standardization is essential, differences in some of these variables, such as growth rates and consequently exposure times, are common in the wild. The growth rates and life span of Many ectotherms vary latitudinally , with slower growth occurring in cooler high latitude environments (e.g., Munch & Salinas, 2009). For instance, the bivalve *Protothaca staminea* reaches a length of 30 mm in 8 years at northern localities along the Pacific Coast of North America, while reaching this length in 3 years at more southern localities (Paul & Feder, 1973). The literature contains numerous examples of species with spatially variable growth rates, generated by both biotic and abiotic factors (e.g., *Mercenaria mercenaria* [Ansell, 1968; Henry & Cerrato, 2007], *Tegula funebralis* [Frank, 1975], *Bembicium vittatum* [Parsons, 1997]). Body size distribution within species also varies between localities: reminiscent of this system, body sizes are left skewed with predominantly large individuals in northern populations of the marine gastropod

Kelletia kelletii (Zacherl et al., 2003), but commonly right-skewed (as with the imaginary southern province) in fossil populations and some recent populations (e.g., Hallam, 1967; Leighton, 2001; Dietl, 2003).

Variation in shell morphology (such as the presence or absence of spines and ridges, variation in average shell thickness, variation in where increased shell thickness is localized, variation in lip thickening and timing of formation of terminal varices) is common in marine mollusks: for example, the marine gastropods *Nucella lapillus*, *Bembicium vittatum*, *Littorina obtusata*, and *Littorina striata* vary in shell shape both between microhabitats and between populations (Parsons, 1997; De Wolf et al., 1998; Trussell & Smith, 2000; Guerre-Varela et al., 2009). The freshwater snail *Physa* also varies its shell shape, apparently in response to varying predatory regimes (DeWitt et al., 2000), as does the terrestrial snail *Satsuma caliginosa*, which has evolved different aperture shapes in the presence of predatory snakes (Hoso & Hori, 2008). Differences in morphology (e.g., shell thickness) have been linked to functional changes in defensive performance (Schindel et al., 1982; Vermeij, 1987; Vermeij, 1993; West & Cohen, 1996; Zuschin et al., 2003), and laboratory experiments have demonstrated that morphological differences may significantly affect survival frequency in predatory encounters. For instance, the queen conch, *Strombus gigas*, grows a more massive shell when raised in the presence of caged lobsters, as compared to conspecifics raised without lobsters present. In a predation experiment in which a lobster was allowed to roam free in tanks with both conditioned and un-conditioned queen conchs of similar size for 24 hours, conditioned snails with more massive shells had significantly higher survival rates (Delgado et al., 2002). Bertness & Cunningham (1981) found that interspecific differences in shell massiveness and sculpture affected predator success in encounters with durophagous crabs.

My assumption that growth rate is invariant throughout ontogeny is not likely to be met in natural systems, as most mollusks (and indeed, most animals [von Bertalanffy, 1957]) decrease growth rates after reaching maturity (e.g., Jones et al., 1989; Allmon et al., 1992; Allmon et al., 1994; Jones & Allmon, 1995; Cadée et al., 1997); however, this assumption allows for simplification of standardization techniques in this example. Methods are easily modifiable for individuals with variable growth, but would require determination of growth rate throughout ontogeny (as in Jones & Allmon, 1995, where stable isotope analyses were used to construct growth profiles). Species that grow determinately to a specific point and cease growth afterwards would require different methods for assessing repair frequency (e.g., *Strombus* sp., *Cassus* sp.): specifically, repairs accumulated after the formation of the terminal lip should not be considered unless time post-maturation can be reliably assessed.

Given the above examples, the preconditions for my *Conchus* system are reasonably conservative, and within the range of variation observed in natural systems. I suspect that systems that significantly differ from these conditions will be the exception, as opposed to the rule, but advocate closer examination of study systems to determine the generality of my assumptions.

Sample Size Considerations

Before assessing the potential magnitude of the standardization problem, an initial consideration for this thought experiment (or any study for that matter) is whether there are sufficient numbers of individuals to allow statistically relevant and powerful conclusions. Kosloski et al. (2008) found that RF with resampling (known for data sets of approximately 200-300 individuals) was highly variable with sample sizes of less than 25; however, above this number, samples accurately captured variation in RF (see Kowalewski, 2002 for further

discussion of sample sizes). A similar study (Forcino, 2011) suggested that samples needed to contain at least 25-50 individuals to accurately capture variation in community paleoecology studies; Olszewski (1999) also recommended a sample size of at least 29 to capture variation in time-averaged assemblages. Given these findings, authors should exercise caution when attempting to draw conclusions from small data sets (e.g., less than 10 individuals in a sample, as in Vermeij, 1987; Chattopadhyay & Baumiller, 2010; Sawyer & Zuschin, 2011). Thus my "dummy" data set was large, and met this basic sample size requirement for all subsequent binnings of data.

PROGRESSIVE STANDARDIZATION

Unstandardized Data

For my hypothetical example, I calculated initially equivalent repair scar frequencies between the northern and southern provinces for the unstandardized raw data: 20% of individuals from both provinces have at least one repair scar on their final whorl, resulting in a repair frequency (RF) of 0.20 (Table 1.1). My data show higher RFs on smaller individuals to the south (Figure 1.3, particularly for the heavily ornamented morphology). Actual literature reports show both higher and lower repair frequencies for juveniles (see Cadée et al., 1997 for examples and discussion of factors that could cause either pattern).

Body Size Standardization

The first standardization was by body size (using individuals 0-200 mm in length, with size bins every 50 mm, Table 1.2). Standardization by size is probably the most prevalent technique (besides standardization by taxon) currently used. Body size may affect both ecological and taphonomic processes (see Zuschin et al., 2003 for a review). If scar accumulation is size-dependent, differences in body size must be considered in analyses (e.g., Vermeij et al., 1981; Cadée et al., 1997; Alexander & Dietl, 2003). When I standardized my data by size, repair frequencies shifted within size classes: in the northern province, RF peaked in the largest size class, whereas in southern populations, RF was highest in the two smallest (0-50 mm and 50-100 mm) size classes. As a caveat, while size standardization does allow assessment of whether or not particular size classes differ in their vulnerability to predators, it does not examine differences in exposure time between size classes. If the implicit assumption of equivalent age across samples does not hold, then repair frequencies will not accurately represent where predation is most heavily concentrated.

Authors discussing heterochrony have also noted the problems inherent in assuming size and age are equivalent (e.g., Godfrey & Sutherland, 1995; Nehm, 2001). A few studies have combined size and age studies to determine the validity of the assumption that size and age are equivalent (e.g., Blackstone & Yund, 1989; Jones & Gould, 1999); however, the difficulty and expense (in the case of geochemical studies) of determining size at age has limited work of this nature (McKinney & McNamara, 1991). Furthermore, even if ontogenetic age can be determined, size standardization does not account for differences in shell shape.

Table 1.1.		Unstandardized	0-50 mm	50-100 mm	100-150 mm	150-200 mm
Northern Populations (*C. i. imaginarius*)	N Individuals	N=500	110	100	130	160
	N Repaired	N=100	12	16	28	44
	RF	**RF=0.20**	***0.109***	***0.16***	***0.215***	***0.275***
Southern Populations (*C. i. imaginarius* and *C. i. ornamentus*)	N Individuals	N=500	150	130	130	90
	N Repaired	N=100	32	32	20	16
	RF	**RF=0.20**	***0.213***	***0.246***	***0.154***	***0.178***

Table 1.2. Unstandardized data and data standardized by size classes.

Shape Standardization

A new standardization technique advocates classifying individuals by shape (Table 1.3), including differences in adaptations such as spinosity, shell thickness, and degree of sculpture (though see Dietl & Hendricks, 2006 for an example of standardization by handedness, an aspect of shape which is particularly important when using predators with preferred chiralities). Mollusks often vary widely in shape within species, and spatially structured variation is common (e.g., Ebling et al., 1964; Hughes and Elner, 1979; Trussell & Smith, 2000; Trussell & Nicklin, 2002).

In the data set, southern populations were split (where both morphs were present) into *C. i. imaginarius* and *C. i. ornamentus* samples. After this correction, RF peaked in *C. i. ornamentus* samples in the 2 smallest size classes, reflecting their relatively higher likelihood of accumulating repair scars within vulnerable smaller size classes. In the two largest size classes, RF remained highest for *C. i. imaginarius* morphs in northern populations. Shifts revealed both a difference in predation vulnerability for the two morphologies in the southern province, and a high predation rate to the north for *C. i. imaginarius* as compared to southern individuals of *C. i. imaginarius.* Even after accounting for size and shape differences, however, one cannot

confidently assume that exposure time has been made equivalent and that samples do not incorrectly conflate size with age (Alexander & Dietl, 2003; Dietl & Alexander, 2009).

Table 1.3.		**Unstandardized**	**0-50 mm**	**50-100 mm**	**100-150 mm**	**150-200 mm**
Southern Populations, corrected by size class   (*C. i. imaginarius.* & *C. i. ornamentus*.)	N Individuals	N=500	150	130	130	90
	N Repaired	N=100	32	32	20	16
	RF	**RF=0.20**	***0.213***	***0.246***	***0.154***	***0.178***
Southern Populations, corrected by morphology  (*C. i. imaginarius*)	N Individuals	N=250	76	60	50	30
	N Repaired	N=36	12	12	6	6
	RF	**RF=0.144**	***0.158***	***0.200***	***0.120***	***0.200***
Southern Populations, corrected by morphology  (*C. i. ornamentus*)	N Individuals	N=250	74	70	80	60
	N repaired	N=64	20	20	14	10
	RF	**RF=0.256**	***0.270***	***0.286***	***0.175***	***0.167***

Table 1.3. Southern population data standardized by morphology. I assume that there should be more large-size class specimens of *C.i.ornamentus*, as they should be more likely to survive predatory attacks. I also assume that they will be approximately twice as likely to accumulate repair scars in smaller size classes, as compared to *C. i. imaginarius*.

Exposure Time Standardization

My RF calculation method uses repair scars accumulated on the final whorl of each specimen, which is presumed to have been deposited over the same amount of time between individuals. If growth rates vary either within individuals at one locality or between localities, comparisons will be inaccurate due to differences in exposure time during the formation of the final whorl (e.g., Cadée et al., 1997). Currently, the effects of differences in growth rates on

repair frequencies have been very rarely accounted for in ecological studies (i.e., Schoener, 1979; Dietl & Alexander, 2009), and are, to best of my knowledge, absent in paleontological studies.

Comparisons were limited to the *C. i. imaginarius* morph. For this data, I am making the simplifying assumption that each size class (0-50 mm, 50-100 mm, and so on) is defined by the addition of one whorl to the shell. With differences in growth rate, this means that individuals to the north would take 3 years to accumulate their first whorl (0-50 mm), whereas individuals to the south would take only 1 year to accumulate their first whorl. This pattern holds for the remaining whorls. I corrected for growth rate in these samples by dividing observed RFs for populations from the northern province by 3, which changes repair frequency estimates for northern populations to a **rate** of repair scars accumulated per year (Table 1.4). Southern populations, which take one year to accumulate each whorl (and to grow through each size class) are already represented by a **rate** of repairs per year. Whereas frequency data represent only a count of how many individuals have repairs, rates additionally provide information as to how rapidly repairs have accumulated. Converting repair data to **rates** of scars accumulated per unit time gives us a much more useful and standard metric; which is robust and transportable from context to context. The term "rate" has occasionally been used incorrectly in a non-time dependent sense in the literature (e.g., Hoffmeister & Kowalewski, 2001; Kowalewski & Hoffmesiter, 2003); I advocate restricting its use only to time-dependent measurements of repair-scar accumulation.

My exposure time conversion was straightforward, because equivalent growth rates throughout ontogeny were assumed: in the real world, this assumption is certainly violated if, for example, growth diminishes in adults as more resources are devoted to fecundity or as

individuals become gerontic (e.g. Bertalanffy, 1957; Goodfriend et al., 1995; Jones & Quitmyer, 1996; Ivany et al., 2003; Henry & Cerrato, 2007; Gentry et al., 2008). In species where growth rates vary throughout ontogeny, calculations should account for the growth rate experienced during the deposition of the final whorl, or interval of the shell over which repair scar accumulation was considered. In practice, this would require the construction of growth curves (see Jones & Allmon, 1995 for an example in *Turritella* spp.). From growth curves, average growth through a size class can be derived and used to normalize exposure time between samples. Standardization shifted repair rates strongly in all size classes to southern populations of *C. i. imaginarius* (Table 1.4).

Producing a standardized repair rate ultimately allows direct comparison of the likelihood of different morphologies accumulating repairs over given time periods. Given the clear utility of this method, I assume that the high cost of geochemical sclerochronology has been the main barrier to general usage (at least, in molluscan studies). In taxa where sclerochronological studies can be combined with geochemical analyses or mark-recapture studies of growth rate, costs might be significantly lowered, whereas in taxa where growth is highly variable within populations and sclerochronology is not possible, costs may be prohibitively high. Additionally, in gerontic individuals where growth is determinate, or in taxa where growth becomes increasingly episodic with age (e.g., Schone, 2008), studies may have to be restricted to certain life stages where growth rate can be accurately assessed. Considering the magnitude of the effect of differences in growth rate on repair scar data, it is hard to justify the general tendency to ignore this particular issue.

Table 1.4.		Unstandardized	0-50 mm	50-100 mm	100-150 mm	150-200 mm
Northern Populations *(C. i. imaginarius)* 	N Individuals	N=500	150	130	130	90
	N Repaired	N=100	32	32	20	16
	RF	**RF=0.20**	***0.213***	***0.246***	***0.154***	***0.178***
Southern Populations (*C. i. imaginarius*) 	N Individuals	N=250	76	60	50	30
	N Repaired	N=36	12	12	6	6
	RF	**RF=0.144**	***0.158***	***0.246***	***0.154***	***0.178***
Exposure time standardization, N. Populations	RF	**RF=0.20**	***0.036***	***0.053***	***0.072***	***0.092***

Table 1.4. Standardized by morphology and rate of scar accumulation. Comparisons are only between *C. i. imaginarius* in the northern and southern populations. My correction gives a rate of repair scar accumulation based on knowledge that *C. i. imaginarius*. Individuals in the south take one year to grow each whorl, whereas individuals to the north take 3 years to grow each whorl.

DISCUSSION

My two province-level samples initially had equivalent repair frequencies (Figure 1.4). Standardization by size (Step 2, Figure 1.4) shifted where repair frequencies were concentrated: RF ranged from 0.11 to 0.28 and peaked in the largest size class in the north, whereas to the south RF ranged from 0.15-0.25 and peaked in the second smallest size class. The next standardization, by shape (Step 3, Figure 1.4), reflected the underlying assumption (supported by laboratory experiments on other organisms) that heavily ornamented individuals would have a higher likelihood of accumulating repair scars in small size classes, where they would be vulnerable to crushing predation but overall more likely to survive, and would have lower repair scar formation in larger size classes, where predators would likely be unable to damage their

shells. This resulted in a shift in RF to higher values within ornamented individuals (*Conchus imaginarius ornamentus*) within the southern province in all but the largest size class. A likely biological interpretation of these results would be that the ornamented morphology entered a partial refuge from predation above 150 mm (see Leighton, 2001 for further discussion of recognizing and interpreting size refuges from predation). The final correction, for exposure time, considered only the non-ornamented morphology, and shifted all peaks strongly to the south, resulting in repair rates ranging from 0.15-0.25 for all size classes to the south vs. 0.04-0.09 to the north.

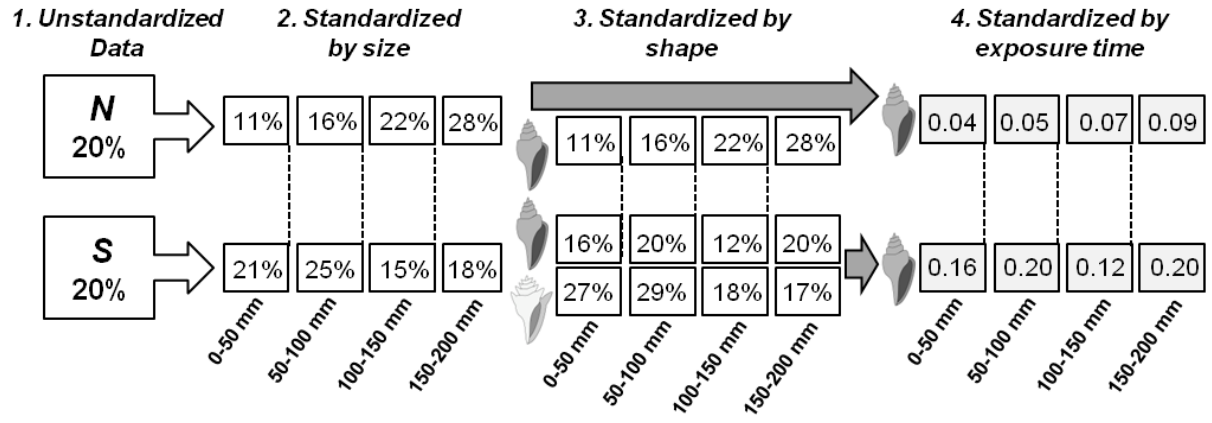

Figure 1.4. Effects of progressive standardization on repair frequency in *Conchus imaginarius*.

While these shifts in RF and repair rate may at first glance seem extreme, assumptions regarding size distributions and vulnerability, relative growth rates, and vulnerability of different morphologies to attack (as well as differences in likelihood of forming repairs scars) range within those found experimentally for modern systems. This exercise is meant to illustrate just how important it may be to carefully account for characteristics of samples that affect repair scar

accumulation (i.e. growth rate, morphology, etc.)—depending on the level of standardization, differences in estimated repair frequency (and rate) varied widely.

Going back to the initial introductory example from De Wolf et al. (1998), standardizing for variation in defensive shell sculpture as well as differences in growth rate between morphologies may make the author's results explicable within their ecological framework of elevated predation to the south. Counter-intuitive trends reported in the literature may result from incomplete consideration of all of the variables that impact repair frequency (taxonomy, habitat, sample size, body size, age, and morphology). Rigorous standardization, though it will require larger sample sizes and increased initial investment in terms of pilot studies of growth rates and morphological variation, would ensure that authors compare ecologically equivalent samples, and that patterns and trends represented ecological signals.

As knowledge increases about how aspects of prey species' life histories (such as morphology, size, and growth rate) affects both predation pressure and the likelihood of accumulating repairs, we must be honest about the limitations of our data when deciding what hypotheses to test, and how rigorously we can test them. Because unsuccessful predation is both ubiquitous and necessary for the evolution of defenses in prey species (Vermeij, 1982), the importance of recognizing it and correctly interpreting RF is essential. RF will only correlate positively with predation intensity (i.e., lethal predation) in a very few cases, where wcomparisons are between similar morphologies, with equal exposure times and size classes. Moody & Aronson's 2007 work on predation in salt marshes is an excellent example of a study where repair scar frequencies have been convincingly shown to represent predation intensity, which may be a result of their fortuitous standardization between sites: habitat, body size, morphology, and a variety of other factors were controlled for, yielding results that matched the

anticipated ecologic signal. More comparative studies would be useful in determining the magnitude of the standardization problem: within-taxon studies should be used to determine when size is NOT equivalent to age, and when shape affects the accumulation of repair scars.

Whereas my primary emphasis is on mollusks, the need for standardization applies broadly to all taxonomic groups. Repair scar analyses have been carried out for a wide range of additional taxa (e.g. ammonites [Bond & Saunders, 1989], brachiopods [Harper et al., 2009; Leighton, 2001], echinoderms [Kowalewski & Nebelsick, 2003], etc.)), several of which may have additional unique challenges associated with repair scar interpretation. Many of the above suggestions should be applicable to these groups; however, authors should consider variation in both ecological and life history traits that affect RF for various taxa.

RECOMMENDATIONS: A BLUEPRINT FOR ACTION

The ultimate goal of standardization is to make sure that the metrics we compare are as ecologically equivalent as possible, which will give us in turn the best chance of capturing signals of interest. As a starting point, I recommend that future studies:

1. Start with taxonomically standardized data (at the lineage/specific level), because the mixtures of adaptive syndromes, habitats, sizes, and exposure time are likely to render comparisons meaningless at the assemblage level
2. Check for size-dependent patterns in structure of data by standardizing for different size classes: if predation is size-dependent only compare like sizes between samples
3. Check for shape-dependent differences in the likelihood of specimens accumulating repairs: if predation is shape-dependent only compare like shapes between samples

4. Verify that exposure time is equivalent between samples: if it is not, convert repair frequencies to repair rates prior to comparing samples

I hope that this paper will spur some much needed discussion on best practices in paleoecology: to date, the issues raised throughout this paper have been largely ignored, potentially at the cost of producing misleading results. Many questions still remain (e.g., Do we need to question results from unstandardized studies? Should we revisit prior work? If we do revisit, and if we start standardizing our data more carefully, what will be the magnitude of the problem?) My example illustrates a relative frequency issue: if large shifts in RF after standardization are common, then we have to reassess how we test (and how we have tested) hypotheses of the importance of predation in evolution. If shifts in RF resulting from standardization are not common, what I have said doesn't matter. I hope this is the case, but given how trait variation affects predation and RF in studied systems, I suspect that standardization will be critical for accurately assessing our hypotheses. Obviously, as with size-and-age work in heterochrony studies, this grand challenge is not going to be easy. Minimally, however, we need to be up front about our assumptions. In some cases, this will undoubtedly limit the conclusions that can be drawn. Ultimately, though, the conclusions that more rigorous standardization will produce should be more robust, and more likely to capture the ecological signals of interest

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REFERENCES

Alexander, R. & G. P. Dietl. 2001. Shell Repair Frequencies in New Jersey Bivalves : A Recent Baseline for Tests of Escalation with Tertiary, Mid-Atlantic Congeners. Palaios, 16(4): 354-371.

Alexander, R. R. and G. P. Dietl. 2003. The fossil record of shell-breaking predation on marine bivalves and gastropods. *in* P. H. Kelley, M. Kowalewski, and T. A. Hansen, eds. Predator-prey interactions in the fossil record. Kluwer Academic/Plenum, New York, p.141-176..

Allmon, W.D., Jones, D.S. & N. Vaughan. 1992. Observations on the biology of *Turritella gonostoma Valenciennes* (Prosobranchia: Turritellidae) from the Gulf of California. The Veliger, 35(1): 52-63.

Allmon, W.D., Jones, D.S., Aiello, R.L., Gowlett-Holmes, K. & P. K. Probert. 1994. Observatios on the biology of *Maoricolpus roseus* (Quoy & Gaimard) (Prosobranchia: Turritellidae) from New Zealand and Tasmania. The Veliger, 37 (3):267-279.

Ansell, A.D. 1968. The rate of growth of the hard clam *Mercenaria mercenaria* (L) throughout the geographical range. J. Cons. Perm. Int. Explor. Mer., 31 (3):364-409.

Arnold. W.S. 1984. The effects of prey size, predator size, and sediment composition on the rate of predation of the blue crab, *Callinectes sapidus* Rathbun, on the hard clam, *Mercenaria mercenaria* (Linné). Journal of Experimental Marine Biology and Ecology, 80(3): 207-219.

Bertalanffy, L.V. 1957. Quantitative laws in metabolism and growth. The Quarterly Review of Biology, 32(3):217-231.

Bertness, M.D. & C. Cunningham. 1981. Crab shell-crushing predation and gastropod architectural defense. Journal of Experimental Marine Biology and Ecology, 50:213-230.

Blackstone, N.W. & P.O. Yund. 1989. Morphological variation in a colonial marine hydroid: a comparison of size-based and age0based heterochrony. Paleobiology, 15 (1):1-10.

Bond, P.N. & W.B. Saunders. 1989. Sublethal Injury and Shell Repair in Upper Mississippian Ammonoids. Paleobiology, 15(4):414-428.

Bookstein. F. L., B. Chernoff, R. L. Elder, J. M. Humphries, Jr., G. R. Smith, and R. E. Strauss. 1985. Morphonletrics in Evolutionary Biology: The geometry of size and shape change, with examples from fishes. Academy of Natural Sciences of Philadelphia Special Publication 15.

Boulding, E.G. 1984. Crab-resistant features of shells of burrowing bivalves: decreasing vulnerability by increasing handling time. Journal of Experimental Marine Biology and Ecology, 76: 201-223.

Cadée, G.C. 2011. Hydrobia as "Jonah in the whale": shell repair after passing through the digestive tract of shelducks alive. Palaios, 26(4):245-249.

Cadée, G. C., Walker, S. E., & K.W. Flessa. (1997). Gastropod shell repair in the intertidal of Bahia la Choya (N . Gulf of California). Palaeogeography, Palaeoclimatology, Palaeoecology, 136: 67-78.

Chattopadhyay, D. and T.K. Baumiller. 2010. Effect of durophagy on drilling predation: a case study of Cenozoic molluscs from North America. Historical Biology, 22: 367-379.

Cintra-Buenrostro, C. 2007. Trampling, peeling and nibbling mussels: an experimental assessment of mechanical and predatory damage to shells of *Mytilus trossulus* (Mollusca: Mytilidae). Journal of Shellfish Research, 26: 221-231.

Cintra-Buenrostro, C.E., Flessa, K.W. & G. Avila-Serrano. 2005. Who cares about a vanishing clam? Trophic importance of *Mulinia coloradoensis* inferred from predatory damage. Palaios, 20:296-302.

Creswell, P.D. & C. L. McLay. 1990. Handling times, prey size and species selection by *Cancer novaezelandiae* (Jacquinot, 1853) feeding on molluscan prey. Journal of Experimental Marine Biology and Ecology, 140:13-28.

Delgado, G. A., Glazer, R. A., & N.J. Stewart. 2002. Predator-Induced Behavioral and Morphological Plasticity in the Tropical Marine Gastropod *Strombus gigas*. Biological Bulletins, 203:112-120.

DeWitt, T.J., Robinson B.W., & D.S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive tradeoff for shell morphology. Evolutionary Ecology Research, 2: 129–148.

De Wolf, H., Backeljau, T., Van Dongen, S., & R. Verhagen. 1998. Large-scale patterns of shell variation in *Littorina striata*, a planktonic developing periwinkle from Macaronesia (Mollusca: Prosobranchia). Marine Biology, 131:309-317.

Dietl, G.P. 2003. Coevolution of a marine gastropod predator and its dangerous bivalve prey. Biological Journal of the Linnean Society, 80: 409-436.

Dietl, G.P. & R.R. Alexander. 1998. Shell repair frequencies in whelks and moon snails from Delaware and southern New Jersey. Malacologia, 39 (1-2): 151-165.

Dietl, G.P. & R.R. Alexander. 2009. Patterns of unsuccessful shell-crushing predation along a tidal gradient in two geographically separated salt marshes. Marine Ecology, 30: 116-124.

Dietl, G.P. & G.J. Vermeij. 2006. Comment on "Statistical Independence of Escalatory Ecological Trends in Phanerozoic Marine Invertebrates". Science, 314 (925e).

Dietl, G.P. & J.R. Hendricks. 2006. Crab scars reveal survival advantage of left-handed snails. Biology Letters, 2: 439-442.

Ebling, F.J., J.A. Kitching, L. Muntz & C.M. Taylor. 1964. The ecology of Lough Ine. XIII. Experimental observations of the destruction of *Mytilus edulis* and *Nucella lapillus* by crabs. Journal of Animal Ecology, 33:73-82.

Elner, R. W., & R.N. Hughes. 1978. Energy maximization in the diet of the shore crab, *Carcinus maenas*. Journal of Animal Ecology, 47(1): 103-116.

Forcino, F.L. 2011. Multivariate assessment of the required sample size for community paleoecological research. Palaeogeography, Palaeoclimatology, Palaeoecology, 315-316:134-141.

Frank, P. W. 1975. Latitudinal Variation in the Life History Features of the Black Turban Snail *Tegula funebralis* (Prosobranchia : Trochidae). Marine Biology, 192: 181-192.

Freestone, A. L., Osman, R. W., Ruiz, G. M., & M.E. Torchin. 2011. Stronger predation in the tropics shapes species richness patterns in marine communities. Ecology, 92(4): 983-93.

Gentry, D.K., Sosdian, S., Grossman, E.L., Rosenthal, Y., Hicks, D., & C.H. Lear. Stable isotope and Sr/Ca profiles from the matine gastropod *Conus ermineus*: testing a multioproxy approach for inferring paleotemperature and paleosalinity. Palaios, 23:195-209.

Godfrey, L.R. & M.R. Sutherland. 1995. Flawed inference: why size-based tests of heterochronic processes do not work. Journal of Theoretical Biology, 172:43-61.

Goodfriend, G.A., Kashgarian, M., & M.G. Harasewych. 1995. Use of aspartic acid racemization and post-bomb ^{14}C to reconstruct growth rate and longevity of the deep-water slit shell *Entemnotrochus adansonianus*. Geochimica et Cosmochimica Acta, 59(6):1125-1129.

Guerra-Varela, J., Colson, I., Backeljau, T., Breugelmans, K. & R.N. Hughes. 2009. The evolutionary mechanism maintaining shell shape and molecular differentiation between two ecotypes of the dogwhelk *Nucella lapillus*. Evolutionary Ecology, 23(2):261-280.

Hallam, A. 1967. The interpretation of size-frequency distributions in molluscan death assemblages. Paleontology, 10(1):25-42.

Hansen, T.A. & P.H. Kelley. 1995. Spatial variation of naticid gastropod predation in the Eocene of North America. Palaios, 10(3):268-278.

Harper, E.M., Pech, L.S. & K.R. Hendry. 2008. Patterns of shell repair in articulate brachiopods indicate size constitutes a refuge from predation. Marine Biology, 156(10):1993-2000.

Henry, K.M. & R.M. Cerrato. 2007. The annual macroscopic growth pattern of the northern quahog (hard clam, *Mercenaria mercenaria* [L.], in Narragansett Bay, Rhode Island. Journal of Shellfish Research, 26(4):985-993.

Hoffmeister, A.P. & M. Kowalewski. 2001. Spatial and Environmental Variation in the Fossil Record of Drilling Predation: A Case Study from the Miocene of Central Europe. Palaios, 16(6):566-579

Hughes, R.N. & R.W. Elner. 1979. Tactics of a predator, *Carcinus maenas*, and morphological responses of the prey, *Nucella lapillus*. Journal of Animal Ecology, 48: 65-78.

Hughes, A.R. & J.H. Grabowski. 2006. Habitat context influences predator interference interactions and the strength of resource partitioning. Oecologia, 149:256-264.

Huntley, J.W. & M. Kowalewski. 2007. Strong coupling of predation intensity and diversity in the Phanerozoic fossil record. Proceedings of the National Academy of Science, 104(38):15006-15010.

Irlandi, E. A. 1994. Large- and small-scale effects of habitat structure on rates of predation: how percent coverage of seagrass affects rates of predation and siphon nipping on an infaunal bivalve. Oecologia, 98:176-183.

Ivany, L.C., Wilkinson, B.H. & D.S. Jones. 2003. Using stable isotopic data to resolve rate and duration of growth throughout ontogeny: an example from the surf clam, *Spisula solidissima*. Palaios, 18:126-137.

Jones, D.S. & W.D. Allmon. 1995. Records of upwelling, seasonality and growth in stable-isotope profiles of Pliocene mollusk shells from Florida. Lethaia, 28(1): 61-74.

Jones, D.S., Arthur, M.A., & D. J. Allard. 1989. Sclerochronological records of temperature and growth from shells of *Mercenaria mercenaria* from Narragansett Bay, Rhode Island. Marine Biology, 102: 225-234.

Jones, D.S. & S.J. Gould. 1999. Direct measurement of age in fossil *Gryphaea*: the solution to a classic problem in heterochrony. Paleobiology, 25(2):158-187.

Jones, D.S. & I.R. Quitmyer. 1996. Marking time with bivalve shells: oxygen isotopes and season of annual increment formation. Palaios, 11(4):340-346.

Juanes, F. & E.B. Hartwick. 1990. Prey size selection in Dungeness crabs: the effect of claw damage. Ecology, 71: 744-758.

Kelley, P.H. & T.A. Hansen. 1993. Evolution of the naticid gastropod predator-prey system: an evaluation of the hypothesis of escalation. Palaios, 8(4):358-375.

Kosloski, M.E., Dietl, G. P., & G.S. Herbert. 2008, Are museum collections adequate to test the escalation hypothesis? : A preliminary case study using the Plio-Pleistocene *Strombus alatus* species complex from Florida. Geological Society of America, Abstracts w/Program, 40(6): 373.

Kowalewski, M. 2002. The fossil record of predation: an overview of analytical methods. *in* Kowalewski, M. and P.H. Kelley, eds., The Fossil Record of Predation. Paleontological Society Special Papers 8**,** Paleontological Society, Yale Printing Service, New Haven, CT, p.3-42.

Kowalewski, M., Flessa, K.W., & J.D. Marcot. 1997. Predatory scars in the shells of a Recent lingulid brachiopod: Paleontological and ecological implications. Acta Paleontologica Polonica, 42:498-532.

Kowalewski, M. & A.P. Hoffmeister. 2003. Sieves and Fossils: Effects of Mesh Size on Paleontological Patterns. Palaios, 18(4/5): 460-469.

Kowalewski , M. & J.H. Nebelsick. 2003. Predation on Recent and fossil echinoids. *In* P.H. Kelley, M. Kowalewski, T.A. Hansen (eds.), Predator–Prey Interactions in the Fossil Record, Kluwer Academic/Plenum Publishers, New York, p. 279–302.

Krug, P.J. 2011. Patterns of Speciation in Marine Gastropods: A Review of the Phylogenetic Evidence for Localized Radiations in the Sea. American Malacological Bulletin, 29: 169-186.

Laurila, A., Lindgren, B., & A.T. Laugen. 2008. Antipredator defenses along a latitudinal gradient in *Rana temporaria*. Ecology, 89:1399-1413.

Leighton, L.R. 2001. New directions in the paleoecology of Paleozoic brachiopods. *In* S. J. Carlson and M. Sandy (eds.), Brachiopods Ancient and Modern: A Tribute to G. Arthur Cooper, Paleontological Society Papers 7, p.185-205.

Leighton, L. R. 2002. Inferring predation intensity in the marine fossil record. Paleobiology, 28(3): 328-342.

Lindstrom, A. & J.S. Peel. 2010. Shell repair and shell form in Jurassic pleurotomarioid gastropods from England. Bulletin of Geosciences, 85(4), 541–550.

McKinney, M.L. & K.J. McNamara. 1991. Heterochrony: the evolution of ontogeny. New York: Plenum Press, 437 p.

Micheli, F. & C.H. Petersen. 1999. Estuarine vegetated habitats as corridors for predator movement. Conservation Biology, 13(4):869-881.

Moody, R.M. & R.B.Aronson. 2007. Trophic heterogeneity in salt marshes of the northern Gulf of Mexico. Marine Ecology Progress Series, 331: 49–65.

Munch, S.B., & S. Salinas. 2009. Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. Proceedings of the National Academy of Science, 106 (33): 13860-13864.

Nehm, R.H. 2001. The developmental basis of morphological disarmament in *Prunum* (Neogastropoda: Marginellidae). *In* M.L. Zelditch (ed.), Beyond Heterochrony: The Evolution of Development. Wiley-Liss, Inc., p. 1-26.

Oji, T., Chigusa, O, & Sato, T. 2003. Increase of shell-crushing predation recorded in fossil shell fragmentation. Paleobiology, 29: 520-526.

Olszewski, T. 1999. Taking Advantage of Time-Averaging. Paleobiology, 25(2):226-238.

Paine, R. T. 1976. Size-Limited Predation : An Observational and Experimental Approach with the *Mytilus- Pisaster* Interaction. Ecology, 57(5): 858-873.

Parsons, K.E. 1997. Contrasting Patterns of Heritable Geographic Variation in Shell Morphology and Growth Potential in the Marine Gastropod *Bembicium vittatum*: Evidence from Field Experiments. Evolution, 51(3):784-796.

Paul, A.J. & H.M. Feder. 1973. Growth, recruitment, and distribution of the littleneck clam, *Protothaca staminea*, in Galena Bay, Prince William Sound, Alaska. Fishery Bulletin, 71 (3): 665-677.

Peterson, C.H. 1982. Clam predation by whelks (Busycon spp.): experimental tests of the importance of prey size, prey density, and seagrass cover. Marine Biology, 66:159-170.

Rex, M.A., Etter, R.J., Clain, A.J. & M.S. Hill. 1999. Bathymetric Patterns of Body Size in Deep-Sea Gastropods. Evolution, 53(4):1298-1301.

Roy, K. & K. K. Martien. 2001. Latitudinal Distribution of Body Size in North-Eastern Pacific Marine Bivalves. Journal of Biogeography, 28(4):485-493.

Sanford, E., Roth, M.S., Johns, G.C., Wares, J.P., & G.N. Somero. 2003. Local selection and latitudinal variation in a marine predator-prey interaction. Science, 300:1135-1137.

Sawyer, J.A. & M. Zuschin. 2011. Drilling predation in mollusks from the lower and middle Miocene of the central Paratethys. Palaios, 26(5):284-297**.**

Schemske, D.W., G.G. Mittelbach, H.V. Cornell, J.M. Sobel, and K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions?. Annual Review of Ecology, Evolution, and Systematics, 40:245-269.

Schindel, D. E., Vermeij, G. J., & E. Zipser. 1982. Frequencies of Repaired Shell Fractures among the Pennsylvanian Gastropods of North- Central Texas. Journal of Paleontology, 56(3): 729-740.

Schmidt, N. 1989. Paleobiological implications of shell repair in recent marine gastropods from the northern Gulf of California. Historical Biology: an International Journal of Paleobiology, 3(1-2):127-139.

Schoener, T.W. 1979. Inferring the properties of predation and other injury-producing agents from injury frequencies. Ecology, 60(6):1110-1115.

Schone, B.R. 2008. The curse of physiology-challenges and opportunities in the interpretation of geochemical data from mollusk shells. Geo-Marine Letters, 28:269-285.

Stachowicz, J., & M. Hay. 2000. Geographic Variation in Camouflage Specialization by a Decorator Crab. The American Naturalist, 156(1): 59-71.

Stafford, E.S. & L.R. Leighton. 2011. Vermeij crushing analysis: a new old technique for estimating crushing predation in gastropod assemblages. Palaeogeography, Palaeoclimatology, Palaeoecology, 305:123-137.

"standardize". Merriam-webster.com. 2012. http://www.merriam-webster.com/dictionary/standardize (9 May 2012)

Trussell, G.C. & M.O. Nicklin. 2002. Cue sensitivity, inducible defense, and trade-offs in a marine snail. Ecology, 83 (6), 1635-1647.

Trussell, G.C. & L.D. Smith. 2000. Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. Proceedings of the National Academy of Science, 97(5), 2123-2127.

Vermeij, G.J. 1977. The Mesozoic Marine Revolution: evidence from snails, predators, and grazers. Paleobiology, 3 (3):245-258.

Vermeij, G.J., Zipser, E. & E.C. Dudley. 1980. Predation in time and space: peeling and drilling in Terebrid gastropods. Paleobiology, 6(3):352-364.

Vermeij, G.J., Schindel, D.E. & E. Zipser. 1981. Predation through geologic time: evidence from Gastropod shell repair. Science, 214(4254):1024-1026.

Vermeij, G. J. 1982. Unsuccessful Predation and Evolution. The American Naturalist, 120 (6):701-720

Vermeij, G.J. 1987. *Evolution and Escalation.* An Ecological History of Life. Princeton University Press, Princeton, N. J., 527 p.

Vermeij, G.J. 1993. A Natural History of Shells. Princeton University Press, Princeton, NJ, 207 p.

Vermeij, G.J. 1999. Inequality and the Directionality of History. The American Naturalist, 153(3): 243-253

Vermeij, G. J. 2002. Evolution in the consumer age: predators and the history of life. *in* Kowalewski, M. and P.H. Kelley, eds., The Fossil Record of Predation. Paleontological Society Special Papers 8**,** Paleontological Society, Yale Printing Service, New Haven, CT, p. 376-393.

Walker, S.E. 2001. Paleoecology of gastropods preserved in turbiditic slope deposits from the Upper Pliocene of Ecuador. Palaeogeography, Palaeoclimatology, Palaeoecology, 166:141-163.

West, K., Cohen, A., & M. Baron. 1991. Morphology and behavior of crabs and gastropods from Lake Tanganyika, Africa: implications for lacustrine predator-prey coevolution. Evolution, 45(3):589-607.

West, K. & A. Cohen. 1996. Shell microstructure of gastropods from Lake Tanganyika, Africa: adaptation, convergent evolution, and escalation. Evolution, 50 (2):672-681.

Yamada, S.B. & E.G. Boulding. 1998. Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. Journal of Experimental Marine Biology and Ecology, 220 (2): 191-211.

Zacherl, D., Gaines, S.D., & S.I. Lonhart. 2003. The limits to biogeographical distributions: insights from the northward range extension of the marine snail, *Kelletia kelletii* (Forbes, 1852). Journal of Biogeography, 30(6):913-924.

Zaton, M. & Salamon, M.A. 2008. Durophagous predation on Middle Jurassic mollusks, as evidenced from shell fragmentation. Palaeontology, 51, 63-70.

Zuschin, M., Stachowitsch, M., & R. J. Stanton Jr. 2003. Patterns and processes of shell fragmentation in modern and ancient marine environments, Earth-Science Reviews, 63:33-82.

CHAPTER 2

GEOGRAPHIC DIFFERENCES IN GROWTH RATE WITHIN THE SPECIES *Busycon carica* GMELIN (NEOGASTROPODA)

ABSTRACT

I compared growth rates for the knobbed whelk, *Busycon carica*, derived from stable isotope sclerochronology, with prior literature estimates obtained using large mark-recapture studies and laboratory rearing. *B. carica* is a long-lived and widely geographically distributed (Cape Cod, MA-Cape Canaveral, FL, United States) predatory marine gastropod that is the target of several fisheries along the eastern coast of the United States. I constructed growth curves for a total of six snails from two populations (two individuals from Long Island, NY and four individuals from Wassaw Sound, GA) and, within the Wassaw Sound population, between two well- and two weakly-ornamented morphs. Estimates of growth rates for Wassaw Sound, Georgia obtained in this study matched estimates from previous studies at that locality. Differences in growth rates observed between this study and prior studies in Virginia and South Carolina likely reflect the influence of laboratory conditions on growth rates, differences in growth rates between genders, and the importance of including incidences of non-growth in models for this episodically growing species.

Stable isotope estimates of growth rate vary within and between populations, with weakly ornamented northern whelks growing more slowly than weakly ornamented southern whelks. Well-ornamented southern whelks have the slowest growth rate. Seasonal growth patterns also vary: northern individuals grow from the spring through the fall and shut down during the winter, whereas to the south, whelks grow in the spring and fall, but shut down growth during both

summers and winters. Differences in the duration of time spent growing annually may result from different feeding patterns between the two localities. Minimum fisheries size limits to the north should be increased, and minimum fisheries size limits to the south should account for differences in growth rate between morphologies.

INTRODUCTION

Many mollusks exhibit latitudinal clines in life history characteristics and associated traits, such as body size and/or growth rate (e.g., Frank, 1975; Olabarria & Thurston, 2003; Munch & Salinas, 2009; Lee & Boulding, 2010). Mollusks, like other ectothermic organisms, generally grow more slowly at cooler temperatures (e.g. Paul & Feder, 1973; Frank, 1975; Belk & Houston, 2002; Olabarria & Thurston, 2003; Angilletta et al., 2004; Heibo et al., 2005; Lee & Boulding, 2010), as a result of lower metabolic levels and thermal constraints on growing seasons. Growth rate varies clinally in several molluscan species in response to abiotic differences (e.g., *Mercenaria mercenaria* [Ansell, 1968; Henry & Cerrato, 2007], *Tegula funebralis* [Frank, 1975], *Bembicium vittatum* [Parsons, 1997]). Abiotic effects on growth are also manifested as seasonal variation within localities for many species, which either reduce or completely shut down growth during annual extremes, including both temperature maxima and minima (e.g., Jones & Quitmyer, 1996; Goodwin et al., 2001; Henry & Cerrato, 2007).

Variation in the degree of development of shell sculpture within species is also common, and may be linked to both abiotic and biotic factors (e.g., Vermeij, 1987, 1993; Palmer, 1990; DeWitt et al., 2000; Trussell & Etter, 2001; Delgado et al., 2002; Hoso & Hori, 2008; Posilovic & Bajraktarevic, 2010). Within populations, different morphologies (e.g., heavily ornamented

vs. weakly ornamented gastropods) may have different growth rates. The production of shell material is energetically expensive (1/3 to ¼ of the total expenditure for growth; Wilbur & Saleuddin, 1983), and as a result, increases in defensive structures are often linked to slower growth rates, as resources must be allocated away from growth along the shell axis to produce materially and energetically expensive shell features (e.g., Palmer, 1990; Trussell & Smith, 2000; Delgado et al., 2002; Trussell & Nicklin 2002).

I used stable isotope geochemistry to investigate differences in growth rate for two populations and two different morphologies of the knobbed whelk, *Busycon carica*, a marine gastropod with episodic and indeterminate growth. Growth rates were compared with estimates from prior studies (mark-recapture, opercular aging, and laboratory rearing). Prior mark-recapture studies have often been skewed towards large, mature individuals, or have not distinguished growth rate between genders; studies classifying individuals by gender have suggested substantial differences in growth trajectories (Kraeuter et al., 1989; Power et al., 2009). My results were compared with prior models to assess the utility of stable isotope schlerochronology for estimating age (Kraeuter et al., 1989; Castagna & Kraeuter, 1994; Eversole et al., 2008; Power et al., 2009).

The use of stable isotopes allowed both estimation of relative growth rates between and within localities, and assessment of seasonality of growth. These results may be useful for explaining both how and why growth rate varies throughout *Busycon*'s range. Furthermore, as *B. carica* is the target of fisheries in several states along the eastern coast of the United States, understanding growth patterns may aid in the management of target populations.

Whelk Fishery

B. carica is extensively fished in all US Atlantic Coast states except Florida to support both regional and international markets (e.g. Davis & Sisson, 1988; Bruce, 2006; Eversole et al., 2008; Power et al., 2009), and the species has been used both for trade and as a food and tool source for hundreds of years (e.g., Moore, 1921; Ceci, 1980; Quitmyer & Reitz, 2006). NOAA's National Marine Fisheries Service reported 2010 landings of 805.3 metric tons (NMFS Fisheries Statistics Division). While annual landings vary with market price, the fishery has generally experienced increased pressure over the past several decades (Leiva & Castilla, 2002). Many fisheries experts believe that the whelk fishery may be highly vulnerable to collapse due to a lengthy time-to-maturity and slow growth of individuals (Davis & Sisson, 1988; Bruce, 2006; Eversole et al., 2008; Walker et al., 2008; Power et al., 2009; Shalack et al., 2011); however, the lack of basic knowledge about the ecology of this species makes the state of the fishery difficult to assess. Minimum size limits are in place in a few fisheries (Bruce, 2006), but vary by state and do not necessarily correlate to a given age or reproductive stage, and many states lack minimum size limits (Bruce, 2006). Studies have suggested that fisheries preferentially remove older, more fecund individuals (Davis & Sisson, 1988; Power et al., 2009), which may greatly diminish the sustainability of current practices. Better estimates of the age and growth rates of *B. carica* are needed to manage this fishery more sustainably.

STUDY SYSTEM

Busycon carica (the knobbed whelk) is a large, common intertidal-to-shallow-marine predatory gastropod that ranges over approximately 1100 km along the eastern coast of North

America, from Cape Cod, Massachusetts to Cape Canaveral, Florida. This region encompasses two distinct biogeographic provinces: the southern Carolinian province, extending south of Cape Hatteras to Cape Canaveral, and the northern Virginian province, which extends north of Cape Hatteras to Cape Cod (Hall, 1964; Hayden & Dolan, 1976; Engle & Summers, 1999; Cook & Auster, 2007). The Carolinian province is strongly influenced by the Florida Current and Gulf Stream and is classified as warm temperate to sub tropical, whereas the Virginian province is strongly influenced by the Virginian Current, and is temperate (Hall, 1964; Hayden et al., 1984; Calder, 1992; Engle & Summers, 1999). Benthic fauna within provinces show a high degree of endemicity, with a distinct break occurring in the vicinity of Cape Hatteras (Calder, 1992; Engle & Summers, 1999), where sharp surface isotherms are present in the winter (Calder, 1992). Whelks in the southern province can migrate annually by as much as 3.8 km (e.g., Magalhaes, 1948; Walker et al., 2004; Walker et al., 2008); however, migration is generally limited to lateral movement along foraging areas and many whelks are recaptured at their release sites in large tagging studies (Walker et al., 2008). Whelks are most abundant on intertidal habitats in the spring and fall; during summers and winters whelks are mostly absent from intertidal areas, remaining buried and inactive in subtidal regions (Magalhaes, 1948; Walker, 1988; Walker et al., 2004; Walker et al., 2008; Shalack et al., 2011). Annual migration and activity data for northern province whelks is currently lacking.

Morphology of *B. carica* varies clinally (Edwards, 1988; Chapter 3), with northern Virginian province individuals characterized by very weakly ornamented shells that are thinner, less spinose, and lack a defensive feature, the tumid ridge (Hollister, 1958), which is present in populations in the southern Carolinian province (Figure 2.1). Southern shells are on average much heavier for a given shell length, representing an increase in shell thickness and

ornamentation (Edwards, 1988), and possess large spines and a tumid ridge. Both weakly and well ornamented morphologies co-exist in all populations south of Cape Hatteras (Edwards & Humphrey, 1981; Chapter 3), though the proportion of individuals displaying the well- and weakly-ornamented morphologies varies. Body size also varies clinally, with larger maximum lengths observed to the north (Abbott, 1974; Kosloski & Dietl, 2011; Chapter 3).

The increased ornamentation present to the south, particularly the tumid ridge, confer an anti-predatory advantage when dealing with durophagous predators such as the stone crab, *Menippe mercenaria*, and spines are likely a defense against gape-limited predators such as the loggerhead turtle, *Caretta caretta* (Kosloski & Dietl, 2011, Chapter 3). The maintenance of well ornamented morphologies in the Carolinian province may restrict linear increases in shell growth, in comparison to weakly-ornamented morphologies at the same locality.

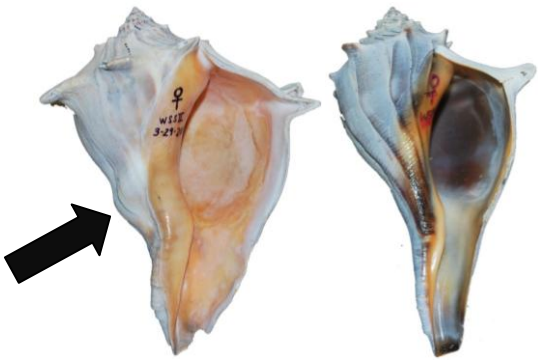


Figure 2.1. Shell on the left (shell length=189.83 mm) shows a typical well-ornamented morphology, with large spines, a thicker shell, and a pronounced tumid ridge (indicated with arrow). Shell on the right (shell length=134.92 mm) shows a weakly-ornamented morphology, with small spines, a thinner shell, and no tumid ridge. Both shells were collected from Cabbage Island, in Wassaw Sound, Georgia.

Prior work on growth rates

Current knowledge of growth rates in *Busycon carica* is limited and occasionally contradictory, with estimates of age varying by almost an order of magnitude; previous studies have estimated maximum ages from a few decades (Power et al., 2009) to close to a hundred years (Eversole et al., 2008). While estimates of age are not well constrained, it is clear that *B. carica* grows episodically and irregularly (Magalhaes, 1948; Dicosimo & DuPaul, 1985), by depositing discrete growth intervals of a few millimeters to several centimeters in as little as a week (Dietl, 2003b). Intervals are then thickened along the interior of the shell (Dietl, 2003b) over the course of several weeks to over a month before subsequent sections are added to the shell, and large temporal breaks may occur between additions of intervals (Magalhaes, 1948). Earlier growth intervals are demarcated from later ones by darkly colored bands, knobs, and/or growth lines in the shell (note banding on right specimen in Figure 2.1, as well as growth lines and knobs). As intervals are not added regularly (as in *Mercenaria mercenaria* or many other bivalves, for example, where annual or seasonal growth lines are useful sclerochronological tools [e.g. Jones, 1988; Jones & Quitmyer, 1996; Goodwin et al., 2001; Ivany et al., 2003; Cledon et al., 2005; Schone et al., 2005; Henry & Cerrato, 2007]) they cannot be counted and used to estimate age.

Prior studies have employed several methods to determine growth rates in *Busycon carica*, including mark-recapture, counting of opercular rings, and laboratory rearing. Mark-recapture studies have been problematic, with negative growth (caused by shell loss) observed for at least some individuals in several studies (e.g., Magalhaes, 1948; Kraeuter et al., 1989; Walker et al., 2008) and sometimes approaching 50% within populations (Eversole et al., 2008). Shell loss may result from self-inflicted damage while feeding, as well as from the actions of

predators. Fisheries, either intentionally targeting whelks or accidentally obtaining them as by-catch, may also break whelk shells (Eversole et al., 2008). Increasing seasonality of growth periods and potentially declining growth rate may reduce annual addition of shell material in older individuals, as has been observed in other marine gastropods (Allmon et al., 1992; Allmon et al., 1994; Goodwin et al., 2003). Diminished growth in gerontic individuals, coupled with shell loss, makes it very difficult to model growth late in ontogeny.

Opercular rings are frequently used to estimate age in marine gastropods, either by embedding the opercula in resin and sectioning it, or by examining the opercula under a light microscope, where dark lines are presumed to represent annual growth intervals (e.g., Kraeuter et al., 1989; Richardson et al., 2005; Power et al., 2009). Laboratory and fisheries studies of *B. carica* and other gastropods have tested the utility of this method with varying results (Kraeuter et al., 1989; Power et al., 2009). One study indicated a tendency to overestimate age using the opercula (by 40% on one 3.6 year old individual and by 12% on one 4.3 year old individual), though over-estimation was minimized by careful examination under a microscope (Kraeuter et al., 1989). Incorrect ages may result from a variety of factors: opercula can be accidentally lost when whelks are captured by either target fisheries or as by-catch, and may also be removed during predatory attacks (Richardson et al., 2005; Power et al., 2009). Opercula may also have multiple nucleation points, which makes assessment of annual rings difficult (Power et al., 2009). Addition of opercular material slows throughout ontogeny, and later rings are difficult to distinguish, making this method challenging to impossible to apply to gerontic individuals (Power et al., 2009).

Only one study (Castagna & Kraeuter, 1994) has raised whelks over a substantial period of time (>10 yrs) in the laboratory, and found fairly linear growth rates after the first year over

the 14 year period of the study. In wild populations, however, where biotic elements (i.e., predation and availability of prey) may be more variable, growth may also be less consistent. Castagna & Kraeuter stated that whelks attained maturity as males at 130 mm and nine years of age, and reversed gender at 172 mm and twelve years of age; however, subsequent work has confirmed that whelks are not protandric hermaphrodites (e.g., Avise et al., 2004) , which makes their results difficult to interpret. Other studies have found variable growth rates between genders, with males reaching smaller maximum sizes and attaining maturity earlier (e.g., Walker et al., 2008; Power et al., 2009). A study conducted in Georgia found that female whelks attained maturity at 100 mm shell length and an age of 6 years (estimated using opercula); males reached maturity at 85-90 mm shell length and 4 years of age (Power et al., 2009).

METHODS

Overview

I applied stable isotope geochemical techniques to wild-collected female individuals of *B. carica* to compare growth rates between the Carolinian and Virginian provinces. My objectives were 1) to assess the accuracy of age estimates obtained as compared to alternate methods (e.g., opercula sectioning, mark-recapture studies, and laboratory rearing); 2) assess whether seasonality of growth rates are apparent; and 3) assess whether the samples analyzed displayed geographic variation in growth rates. I also examined intrapopulational differences in growth using morphologically disparate shells to determine if adaptive costs (diminished growth rates) were associated with producing and maintaining the thicker shells, longer spines, and pronounced tumid ridge seen in many individuals to the south. Prior work has not investigated

whether there are adaptive trade-offs associated with producing the heavily armored morphotype of *B. carica*, though reduced linear growth rates and/or reduced reproductive ability are associated with defensive morphologies in other gastropod genera (e.g., *Nucella*, Palmer, 1990; Geller, 1990; *Littorina*, Trussell & Nicklin, 2002; *Strombus*, Delgado et al., 2002).

Stable Isotope Analysis

Oxygen has two (relatively) common isotopes, ^{16}O and ^{18}O. Proportions of ^{18}O to ^{16}O vary seasonally, with the amount of ^{18}O inversely proportional to water temperature. This generates annual curves in the relative abundance of these two isotopes (e.g., Jones, 1988; Jones & Quitmyer, 1996; Goodwin et al., 2001; Ivany et al., 2003), which can be used to infer age. Stable isotope geochemistry also allows the determination of the temperatures at which shell addition occurs, as temperature can be calculated from the relative abundance of ^{18}O to ^{16}O incorporated into the shell at any point. Stable isotope sclerochronological studies therefore may provide new information that cannot be obtained via other aging methods (such as seasonality of growth, and temperatures at which individuals add to their shells).

I completed serial stable isotopic analysis of oxygen isotopes for six shells (two from Long Island, New York and four from Wassaw Sound, Georgia representing two each of the weakly- and well-ornamented morphologies present at that locality; see Table 2.1 for description of shells), as well as additional carbon isotopes for five of the shells, to estimate age and growth rates. All shells were from female whelks, to minimize the effect of differences in growth rates between genders (e.g., Magalhaes, 1948; DiCosimo and DuPaul, 1985; Power et al., 2009). Shells were obtained from: 1.) museum collections (Paleontological Research Institution, Ithaca, NY) where individuals were collected live, sexed and then killed with soft tissue removed (West Neck Point, Shelter Island, Long Island, NY; collected by G.P. Fisher in 1971; LIN I, LIN II); 2.)

live-collected individuals from Wassaw Sound, Georgia (WSN I) that were sexed in the lab, and then frozen and cleaned to remove soft tissue; 3.) live-collected or recently dead individuals from a large mark-recapture study done by R.A. Walker at Wassaw Sound, Georgia (WSN II, WSS II, WSS III).

Sample ID	Locality	Date Collected	Morphology	Samples per shell	Shell length
WSN I	Wassaw Sound, GA	6/16/2009	Weakly armored	54	122.76 mm
WSS II	Wassaw Sound, GA	3/29/2006	Well armored	86	189.83 mm
LIN I	West Neck Point, Long Island Sound, NY	6/27/1971	Weakly armored	90	167.3 mm
WSN II	Wassaw Sound, GA	3/21/2006	Weakly armored	65	134.92 mm
LIN II	West Neck Point, Long Island Sound, NY	6/27/1971	Weakly armored	56	155.14 mm
WSS III	Wassaw Sound, GA	Feb-Apr. 2006	Well armored	70	135 mm

Table 2.1. Sample IDs, localities, date collected, morphology, samples per shell, and shell lengths for shells used in stable isotope analysis.

Shells were scrubbed prior to sampling and air dried. Powdered aragonite samples (approximately 0.1-0.3 mg) were collected serially from the outer shell layer, starting at the apex and moving towards the aperture, using a hand held dental drill with a small dental burr. Growth intervals were demarcated by the presence of dark colored bands on the shell (particularly in the earliest parts of the shell), strong growth lines, and/ or the presence of a knob or spine (see Table 2.1 for number of samples analyzed per shell). Because growth intervals are laid down over discrete time periods (Magalhaes, 1948; Dicosimo & DuPaul, 1985), only one sample was collected per interval for most intervals. On one specimen (LIN I), several later growth intervals were sampled multiple times to check the assumption that deposition of individual growth intervals occurred rapidly. Samples were collected in linear transects that paralleled prior and

subsequent growth bands (Figure 2.2.). The dental drill was cleaned between samples using isopropyl alcohol and compressed air, and shells were cleaned between each sample with compressed air to minimize cross contamination.

Figure 2.2. Specimen LIN I. Black dots mark every five samples.

Samples were sent to Keck Paleoenvironmental and Environmental Stable Isotope Lab at the University of Kansas for analysis. At the Keck Lab, samples were loaded into stainless steel boats and roasted under vacuum at 180° C for one hour to release any volatile compounds. Following this procedure, samples were transferred to glass vials and reacted for 4 minutes under vacuum with phosphoric acid at 75° C. Released CO_2 was trapped and transferred to an isotope ratio mass spectrometer (Kiel Carbonate Device III + Finnigan MAT253) instrument, where it was measured versus a calibrated CO_2 reference tank. NBS-18 Carbonatite (NIST Ref. Mat. 8543), NBS-19 Limestone (NIST Ref. Mat. 8544) and an internally calibrated calcite standard were analyzed at intervals throughout the analysis and used to generate a 3 point calibration curve, and data were calibrated against this curve and reported verses the VPDB scale. Precision for $\delta_{18}O$ and $\delta_{13}C$ were respectively better than 0.06% and 0.03%. For samples WSN II, WSS III, and LIN II, only every other sample was analyzed early in ontogeny (WSN II: samples 1-36, WSS III: samples 1-30, LIN II: samples 1-40) as resolution obtained at this sampling density for specimens WSN I, WSS II, and LIN I showed that more dense sampling would not additionally

improve the accuracy of the isotope profiles. Values for δ18O and δ13C throughout ontogeny were plotted against each other for each specimen to assess whether values co-varied.

Age and growth rate determination

I counted annual maximum/minimum cycles in the oxygen isotope curves to infer ontogenetic age for the six shells. Oxygen isotope curves were compared with the carbon isotope curves for additional clarification when necessary, as carbon isotopes also often show annual cycles (e.g. Buick & Ivany, 2004)..

To determine growth rates, I fit linear regressions of shell length (from the apex to the tip of the siphonal canal) versus shell width (measured on the final whorl at the aperture, exclusive of spines) to collections of individuals from the two populations (Wassaw Sound, Georgia; West Neck Point, Long Island Sound, NY), with Wassaw Sound, Georgia specimens additionally categorized by morphology (well and weakly armored). I calculated R^2 values for length and width, and used the best fit equation from the linear regressions to estimate shell length using a given shell width, which was measured on shells at samples corresponding with maximum and minimum oxygen isotope values using digital calipers, to the nearest tenth of a millimeter. Later whorls overlap earlier whorls over most of the shell; however, the shoulder remains visible. This procedure allowed accurate measurement of shell width throughout ontogeny, and therefore accurate estimation of associated shell length. From length vs. age estimates, I constructed individual growth curves.

Growth and seasonality

To determine the range of temperatures during which the sampled whelks added new intervals to their shells, I obtained temperature and salinity data for the two localities (West Neck Point, Long Island and Wassaw Sound, Georgia). Seasonality of growth can be determined by

comparing monthly temperature averages for different localities to estimates of the temperature at which the shell was precipitated, which can be solved for using Grossman and Ku's (1986) equation relating temperature to δ18O from shell aragonite:

$$T(°C)=20.6-4.34[\text{shell}\delta^{18}O_{PDB}-\text{seawater}\delta^{18}O_{locality}]$$

This equation is used in combination with the following equation, (e.g., Fairbanks, 1982; Ivany et al., 2003) which adjusts for salinity to solve for ocean water δ18O values at specific localities:

$$\text{Seawater } \delta^{18}O_{SMOW}= [0.258*\text{salinity}(‰)]-9.14$$

Annual temperature variation at the two localities (West Neck Point, Long Island and Wassaw Sound, Georgia) was obtained from Georgia's Department of Natural Resources (DNR), which conducts monthly sampling in Wassaw Sound, and from NOAA's National Data Buoy Center (NDBC, http://www.ndbc.noaa.gov/). For Wassaw Sound, Georgia, data were obtained from 1990 to 2012 from Station 1152, on Dead Man's Hammock. For Long Island Sound, temperature data were obtained from Buoy #44025. Buoy #44025 is located in 40 meters of water, 33 miles south of Islip, Long Island, New York. Data for Buoy #44025 was available from 1975-1980 (Figure 2.3a). Water temperature data from the National Data Buoy Center were used to construct annual temperature curves and estimate monthly temperature averages. Monthly temperature averages were compared to maximum and minimum temperature values estimated from the isotopic profiles of the whelks to determine whether shell growth occurred over the full range of annual temperatures at the two localities.

I used salinity values from DNR monitoring data (ranging from 1990-2012) to calculate δ18O for Wassaw Sound. Monthly salinity readings were obtained from Station 1152 (Figure

2.3b., Dead Man's Hammock) which is approximately a mile from Cabbage Island, and averaged 27.7‰, +/- 3.1‰. Salinity in Peconic Bay in the vicinity of Shelter Island is strongly influenced by freshwater groundwater input (Laroche et al., 1997), and observations from earlier studies averaged 28‰, with a range of +/-1‰ (Bruno et al., 1980; LaRoche et al., 1997).

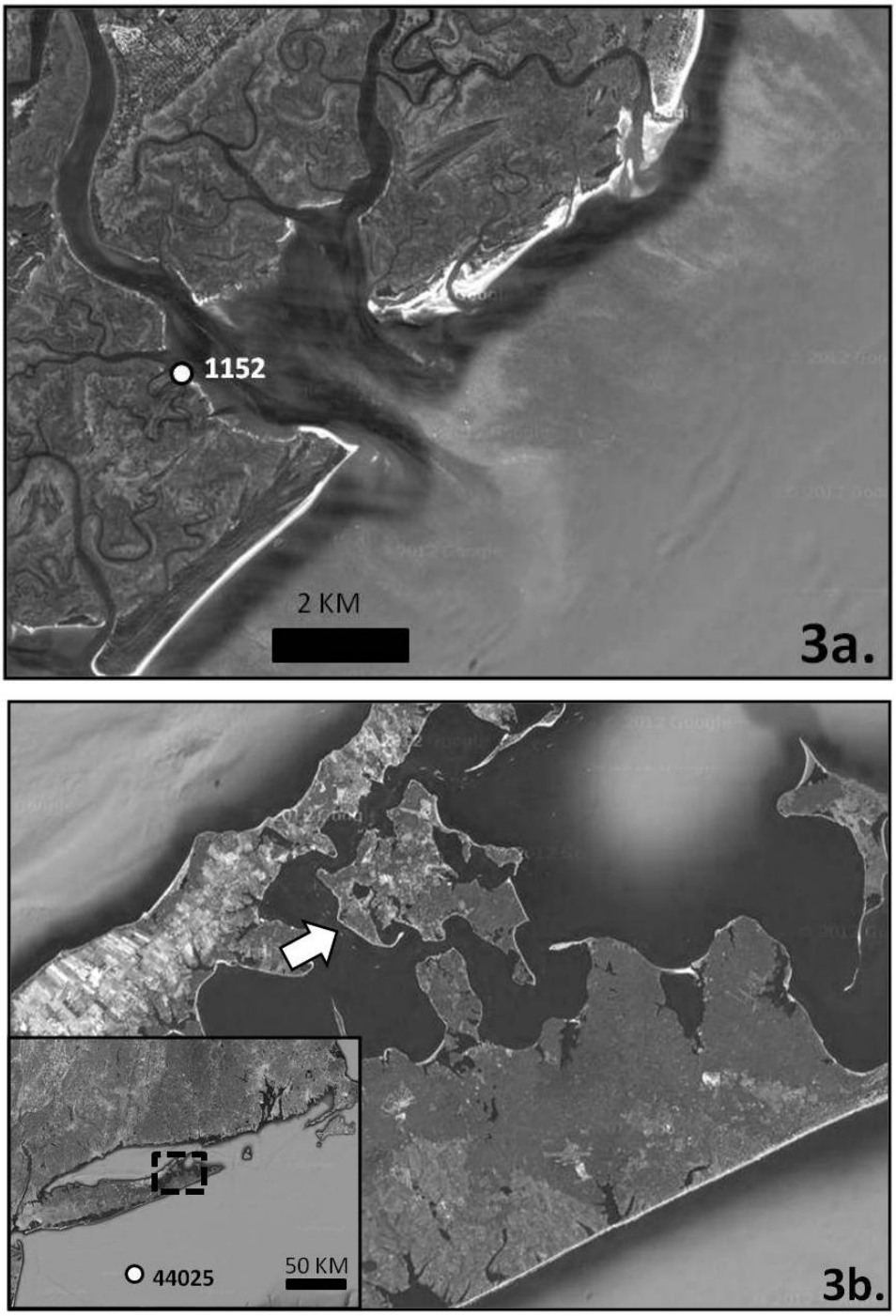
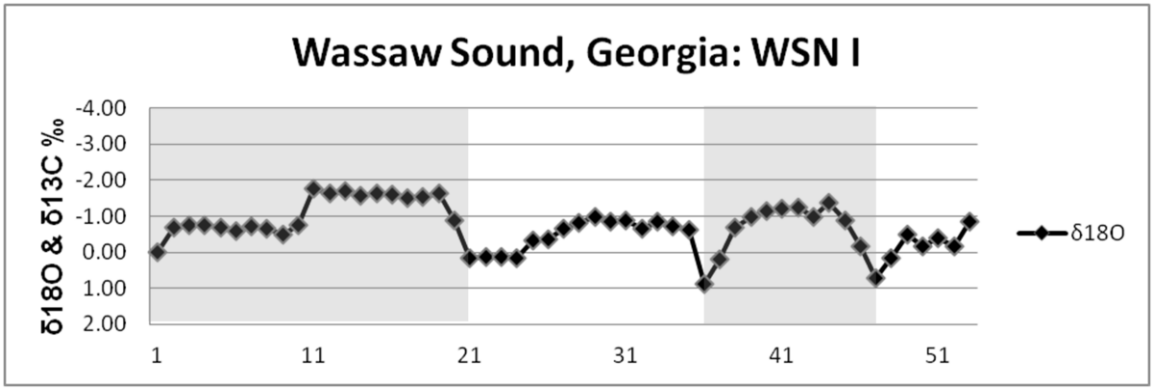


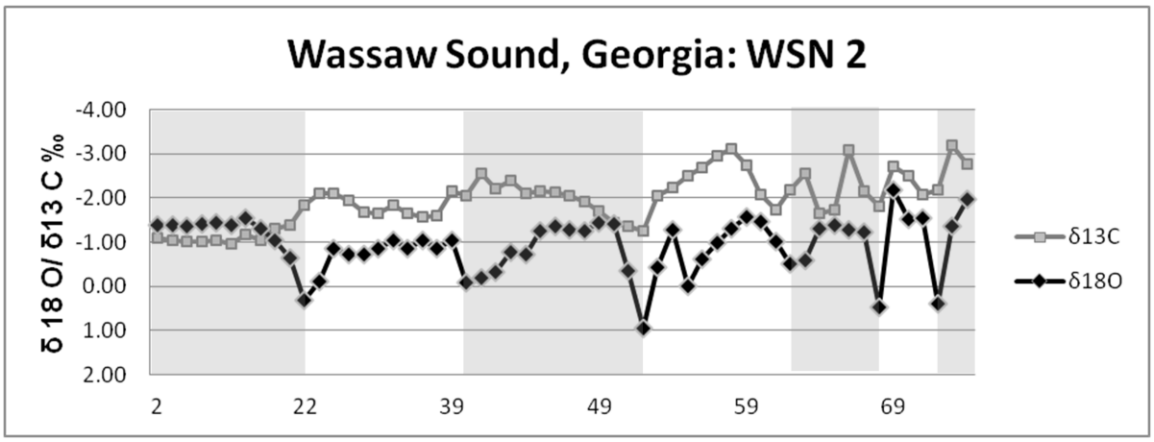
Figure 2.3. 2.3a ("Wassaw Sound, Georgia") shows localities where whelks, salinity, and temperature data were collected in Wassaw Sound. Figure 2.3b. ("Peconic Bay, Long Island") shows sampling localities for Long Island: small inset map has location for Buoy 44025; arrow on larger map indicates collection locality for whelks. Both images Google Maps, 2012.

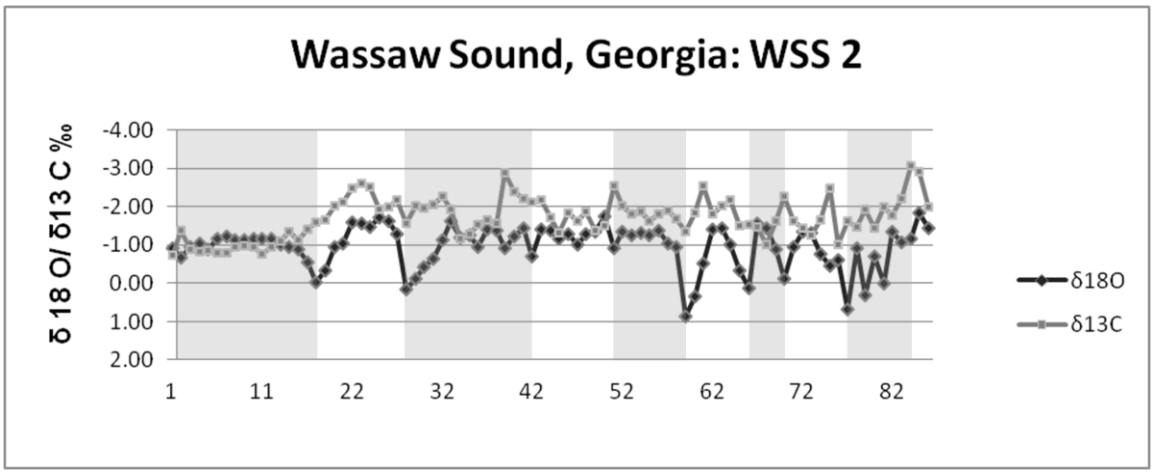
RESULTS

Stable Isotope Analysis

Oxygen isotope profiles showed strong cyclicity throughout ontogeny for four shells (WSN I, WSN II, LIN I, and LIN II), and initially strong cyclicity early in ontogeny followed by weaker cyclicity after the first 3 years for the other two shells (WSS II and WSS III) (Figure 2.4.). Oxygen isotope values varied for the four Georgia specimens between 1.00‰ to -2.18‰; Long Island specimens varied from -0.62‰ to -3.48‰. Ranges of variation were overall similar, extending over approximately 3‰ (Table 2.2.). Variation between sequential samples became more pronounced later in ontogeny, particularly for well-ornamented Wassaw Sound specimens (WSS II and WSS III). Multiply sampled growth intervals from LIN I showed variation in carbon and oxygen isotope values (Table 2.3.), though differences in isotope values were less than 0.5‰.


Wassaw Sound, Georgia: WSN I
δ18O & δ13C ‰
-4.00
-3.00
-2.00
-1.00
0.00
1.00
2.00
1
11
21
31
41
51
δ18O


Wassaw Sound, Georgia: WSN 2
δ 18 O/ δ13 C ‰
-4.00
-3.00
-2.00
-1.00
0.00
1.00
2.00
2
22
39
49
59
69
δ13C
δ18O


Wassaw Sound, Georgia: WSS 2
δ 18 O/ δ13 C ‰
-4.00
-3.00
-2.00
-1.00
0.00
1.00
2.00
1
11
22
32
42
52
62
72
82
δ18O
δ13C

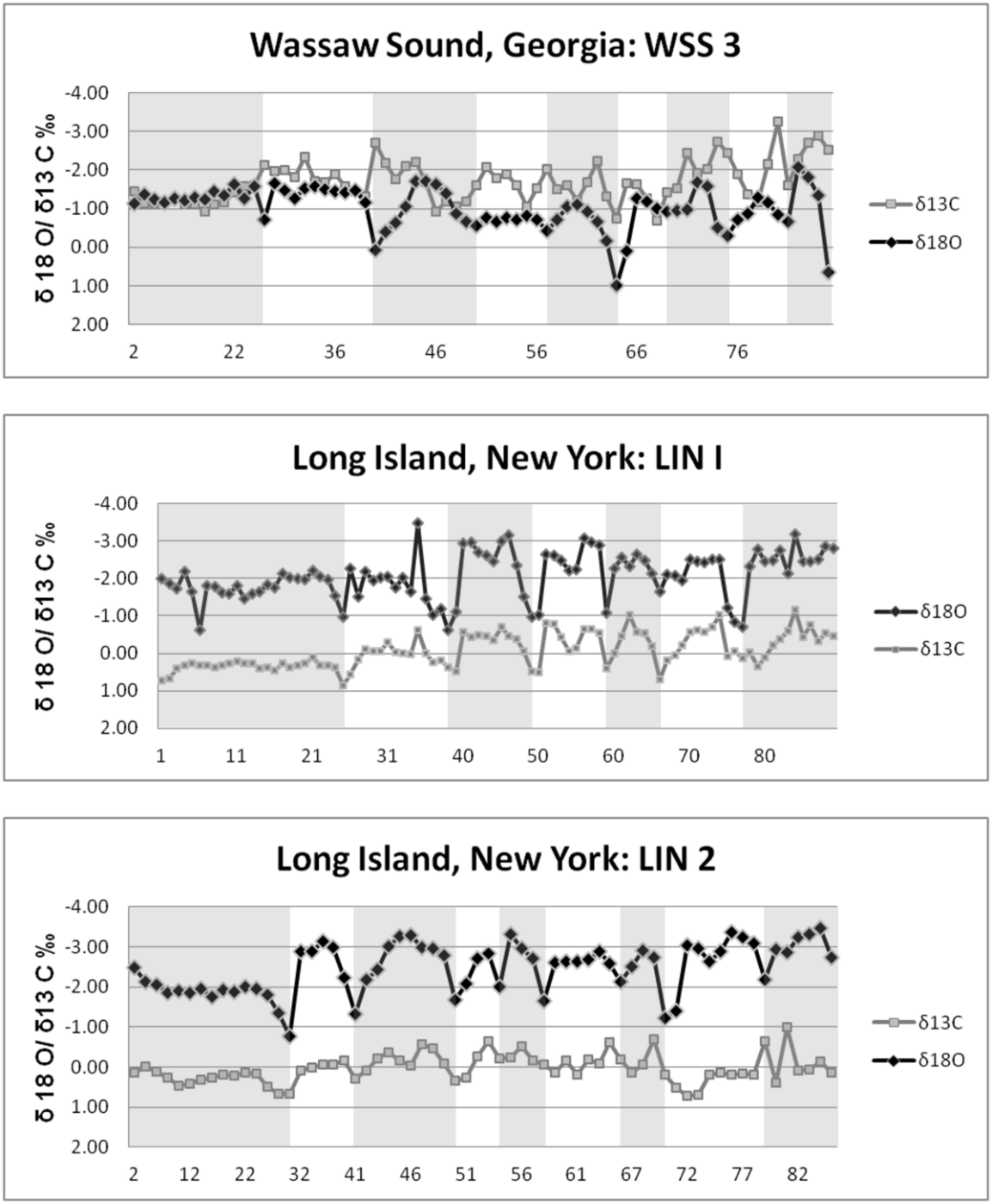


Figure 2.4. Stable isotope profiles. X axis shows sample number, from apex (on left) to aperture (on right). Y axis shows variation in δ18O, with lighter (warmer) values higher on the axis, and variation in δ13C. Alternating grey and white intervals mark consecutive years, representing winter-to-winter (positive δ18O values) time periods. First grey band on left marks time to first winter, which may not represent a full year.

	δ18O, ‰			δ13C, ‰		
	Average	**Maximum**	**Minimum**	**Average**	**Maximum**	**Minimum**
WSN I	-0.72	0.87	-1.78			
WSN II	-0.94	0.94	-2.18	-1.95	-0.97	-3.19
WSS II	-0.97	0.85	-1.83	-1.71	-0.74	-3.08
WSS III	-1.03	1.00	-2.08	-1.69	-0.70	-3.25
LIN I	-2.07	-0.62	-3.48	-0.07	0.86	-1.15
LIN II	-2.48	-0.77	-3.46	0.02	0.72	-1.00

Table 2.2. Isotope minima, maxima, and averages.

	Samples 63, 64, and 65	Samples 80, 81	Samples 88, 89
δ18O	-2.63‰, -2.47‰, -2.12‰	-2.46‰, -2.49‰	-2.51‰, -2.87‰
δ13C	-0.57‰, -0.54‰, -0.18‰	0.11‰, -0.22‰	-0.33‰, -0.54‰

Table 2.3. Variation in δ18O and δ13C for a series of samples collected within individual growth intervals for LIN I.

Carbon and oxygen isotope values did not co-vary significantly for three of the Georgia shells: WSN II (R^2=0.01), WSS II (R^2=0.02), or WSS III (R^2=0.061). While both carbon and oxygen appeared to vary annually, peaks were slightly out of phase throughout the isotope profiles. Variation in δ13C ranged from -0.97‰ to -3.25‰ for all Georgia specimens, and was slightly more negative on average for the weakly ornamented specimen (WSN II, -1.95‰ vs. -1.71‰ and -1.69‰ for well-ornamented specimens). In contrast, carbon and oxygen isotopes co-varied weakly but positively for the Long Island shells: LIN I and LIN II (R^2=0.52 & R^2=0.17, respectively). For all Long Island specimens, δ13C ranged from a maximum value of 0.86‰ to a minimum value of -1.15‰. Carbon isotope profiles for the Long Island whelks are additionally positively shifted 1.5‰ to 2.0‰ relative to the Wassaw Sound specimens. Older specimens from Wassaw Sound (particularly WSN II and WSS II) have erratic isotope profiles later in ontogeny that shift rapidly between depleted and heavier values. Long Island specimens tended to have longer series of depleted (more negative) samples, particularly later in ontogeny.

Age and growth rate determination

Sample	Inferred Ontogenetic Age
WSS II	7-8 years of age
WSS III	9 years of age
WSN I	3.5 years old
WSN II	6-6.5 years old
LIN I	6.5 years old
LIN II	8-9 years old

Table 2.4. Inferred ages of whelks, determined by counting cycles in isotope profiles.

The oldest individual (WSS III) shows 9 full cycles in its oxygen isotope profile, and the youngest individual (WSN I) shows only 3.5 annual cycles (Table 2.4). After sample 77, clear annual cycles are no longer evident in WSS II's isotope profiles, as values fluctuate up and down from sample to sample. For WSS III, three annual curves are evident initially in the oxygen isotope profile; annual variation in the carbon curve was used to infer cycles four and five as oxygen samples 50-60 show only low amplitude variation. WSN II's oxygen isotope profile shows 6 annual cycles; LIN I and LIN II show respectively 6.5 and 8-9 full cycles.

Linear regressions of shell length vs. shell width were highly correlated for all populations and for different morphologies within Wassaw Sound, with R^2 values ranging from 0.90-0.97 (Figure 2.5). Growth rates differed both between the northern and southern provinces, and between the two morphologies present south of Cape Hatteras (Figure 2.6). Weakly armored individuals from the southern province showed the most rapid growth, reaching lengths of approximately 85 and 95 mm after three years. Individuals from Long Island grew at a slightly slower rate, reaching lengths of 70 and 80 mm at approximately 3 years of age. Heavily armored individuals from the southern province had the slowest growth rates, taking three years to attain lengths of 45 and 70 mm. Growth later on in ontogeny becomes more difficult to resolve, as (particularly with the heavily ornamented WSS specimens) variation becomes at times both more erratic and occasionally less pronounced; however, by year 5 for WSN II and years 6 and 7

respectively for WSS II and WSS III growth appears to be leveling off. This trend of diminished growth later in ontogeny is not evident for the two specimens from Long Island.

Estimates of growth rates derived from prior studies (Figure 2.6) vary geographically, with higher growth rates observed to the south (Walker et al., 2008). Power et al.'s (2009) growth model for female whelks from Wassaw Sound, Georgia closely tracks my growth estimates for well-ornamented females. Similarly, Kraeuter et al.'s (1989) growth curve, which was derived from laboratory-reared individuals in Virginia, corresponds well with my estimates of growth rates for whelks from New York, particularly early in ontogeny. Later in ontogeny (after year 4), Kraeuter's growth curve shows slower rates. In contrast, Eversole et al.'s (2008) growth model, based on a mark-recapture study of whelks in South Carolina, deviates substantially from both other prior studies and my estimates of growth rate.

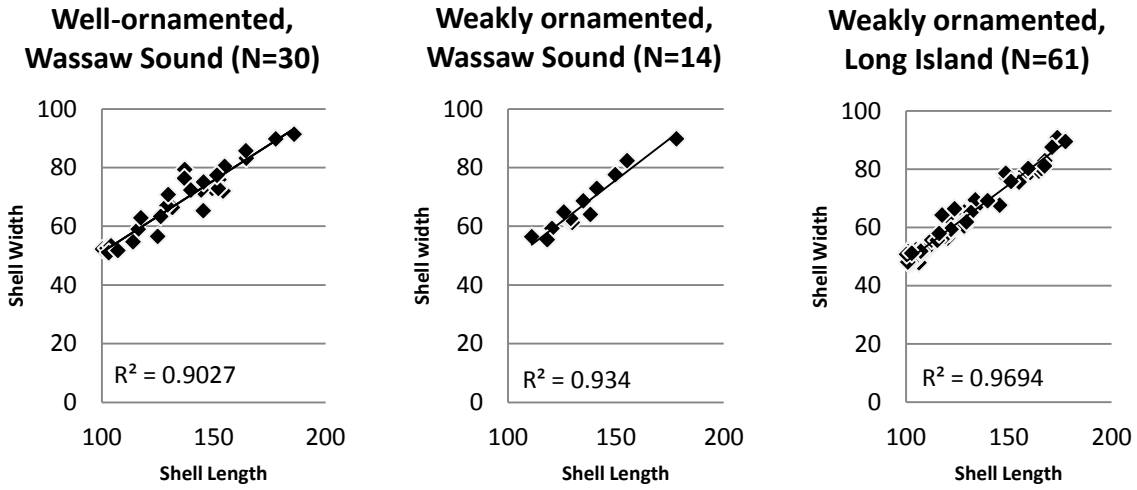


Figure 2.5. Length vs. width plots and R^2 values for different populations and morphologies.

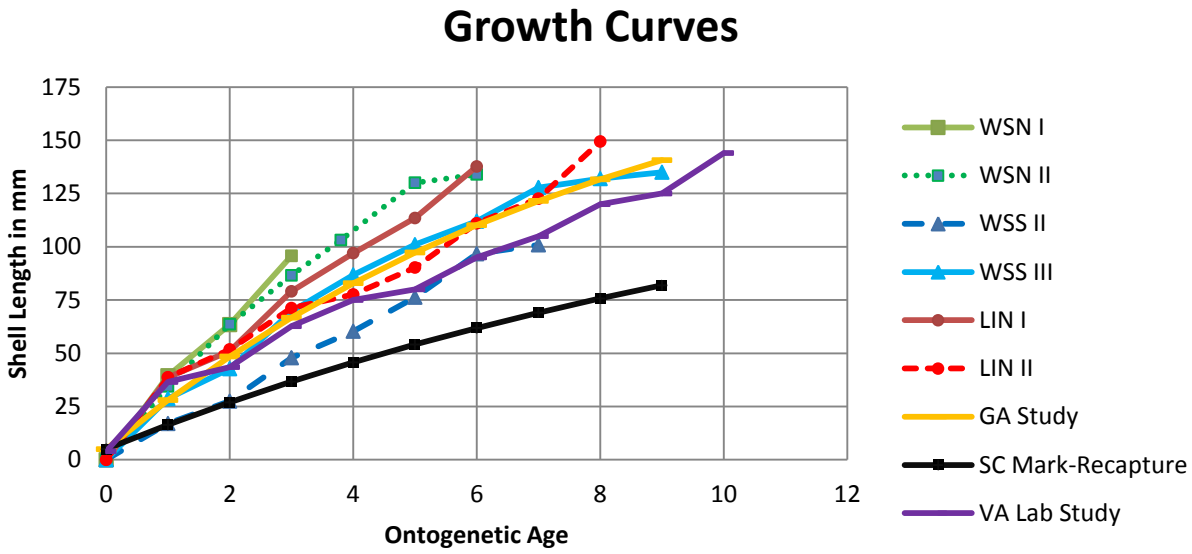


Figure 2.6. Growth curves for six sampled individuals, as well as three literature estimates of growth rates obtained from prior studies (GA Mark-Recapture from Power et al., 2009, SC Mark-Recapture from Eversole et al., 2008, and VA Lab Study from Kraeuter et al., 1989).

Growth and seasonality

Water temperature in Wassaw Sound, Georgia varies annually over approximately 20° C, from a low of 11° in the winters to a high of 31° in the summers. Only ~12°C (Epstein et al., 1953) variation is represented by the 3‰ variation in the Wassaw isotopic profiles. Solving for water temperature with mean ocean water values corrected for salinity, the maximum temperature that growth occurred at was 21.4°C (range from 18-24°C) and the minimum temperature at which growth occurred was 7.6°C (range between 4-11°C), with a possible high (ranges were calculated to account for one standard deviation in salinity at this locality, which is 3.1‰). Most growth (77-90% of sampled intervals, depending on the shell sampled) occurred between δ18O values of -0.25‰ to -1.75‰, which corresponds to temperatures ranging from 13.0°C to 19.5°C. Monthly temperatures in Wassaw Sound generally exceed 13.0°C by late February or early March, rising above 19.5°C in April and remaining above 19.5°C until late October or November (Table 2.5). Temperatures drop below 13.0°C in January and for much of

February, suggesting that whelks at this locality add to their shells predominantly in November and December and in late February through early April. New shell growth rarely occurs from May to late October.

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
11.4	13.2	15.3	20.6	24.2	29.1	31.6	29.3	28.1	22.7	18.5	14.1

Table 2.5. Average monthly water temperature, Station 1152, Dead Man's Hammock, Wassaw Sound, Georgia DNR. Gray's Reef. National Data Buoy Center, NOAA.

Water temperature in Long Island Sound varies annually over approximately 18° C, from a low of 5° C in the winter to a high of approximately 23° C in the summer (Table 2.6). Isotopic profiles for the Long Island shells only vary over about 12°C (3‰ for δ18O), which suggests that northern province whelks may also seasonally shut down growth. After solving for mean ocean water values (corrected for salinity, using a value of 28‰), I determined that shell material was added between minimum and maximum values of 15.0°C and 27.4°C. Addition of most new shell material for Long Island Sound whelks occurred predominantly (84-92% of sampled intervals) between -1.5‰ and -3.5‰, corresponding to a temperature range of 18.8-27.5°C. Long Island Sound whelks likely start adding shell material in late June, and continue to do so through late September or early October.

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
7.56	5.38	5.19	6.67	10.76	16.49	21.63	22.7	20.94	15.45	12.85	10.49

Table 2.6. Average monthly water temperature, Long Island Sound, Buoy #44025, National Data Buoy Center, NOAA.

DISCUSSION

Stable isotope analysis proved useful for determining both seasonality of growth, andfor assessing growth rates within localities. Most differences between growth curves from study versus prior studies likely reflect different treatment of data (discussed below). Growth rates between morphologies varied substantially, suggesting a possible adaptive cost associated with producing the well-ornamented morph. Differences in growth curves for the weakly-ornamented morph between the Virginian and Carolinian province was not as large as expected. Timing of growth varies between localities, with individuals to the north only shutting down growth in the winter, as opposed to southern whelks, which halt growth in both winters and summers. This pattern may account for the similar growth rates observed between LIN I, LIN II, WSN I, and WSN II. Factors possibly affecting this are discussed below.

Age and growth rate determination

Prior studies (using mark-recapture and opercular data) have indicated that growth rates vary between busyconine whelks from Virginia and Georgia (Castagna & Kraeuter, 1994; Walker et al., 2008; Power et al., 2009); the results reported here confirm this general latitudinal trend. This study additionally revealed variation in growth rates between morphologies within the southern province, which had not been noted in prior work.

Highest growth rates were recorded for the weakly ornamented southern individuals, WSN I and WSN II, which attained shell lengths of 100 mm in approximately 4 years. Castagna and Kraeuter (1994) did not observe female whelks laying egg cases until they were greater than 12 years old in their laboratory study in Virginia, suggesting that maturity is reached at a substantially later age in Virginia than to the south. My Long Island whelks were 6 to 7

years old and 155-167 mm long and so had not reached reproductive age. Differences in maximum body size have been noted for *B. carica* (Abbott, 1974), with larger sizes reported to the north. Larger body sizes in the northern province likely result from delayed maturation, which would allow resources to be allocated entirely to growth, as opposed to reproduction, over a longer time span for northern province populations.

All growth curves displayed initially rapid growth, followed by diminished growth later in ontogeny; this pattern has been noted in prior studies of *B. carica* (Castagna & Kraeuter, 1994; Eversole et al., 2008) as well as in other marine mollusks (e.g. *Placopecten magellanicus,* Krantz et al., 1984; *Tridacna maxima,* Jones et al., 1986; *Spisula solidissima* and *Placopecten magellanicus,* Krantz et al., 1987; *Mercenaria mercenaria,* Jones et al., 1989; *Maoricolpus roseus,* Allmon et al., 1994; *Spisula solidissima,* Ivany et al., 2003; *Conus ermineus,* Gentry et al., 2008). None of the individuals appeared to reach a growth maxima, and although three southern specimens (WSN II, WSS II, and WSS III) appeared to show a reduction of growth rates later in ontogeny (around 5-6 years of age), the short temporal duration of these records (the maximum recorded age for any individual was around 7) limits confidence in this trend of diminished growth. Specimens from Long Island (LIN I or Lin II), however, did not show a trend of diminished growth later in ontogeny. This may be related to differences in age at maturity: Wassaw Sound whelks had likely reached reproductive maturity, whereas Long Island Sound whelks may have still been 5-6 years from attaining reproductive age. Towards the end of some of the Wassaw Sound isotope curves, annual variation became more difficult to distinguish, as the addition of shell material slowed to a few discrete intervals per year. Additional techniques may be needed to resolve growth rates and absolute age in gerontic

individuals, as isotope curves are likely to use resolution in the largest samples with increasing age regardless of locality.

Comparisons with prior studies

Growth rates obtained in this study were compared with estimates of growth from three prior studies (Figure 2.6): one conducted in South Carolina (Eversole et al., 2008), one conducted in Virginia (Kraeuter et al., 1989), and one conducted in Wassaw Sound, Georgia (Power et al., 2009). As mentioned briefly in Results, growth models derived from Power et al.'s 2009 study (using opercular estimates of age from wild-caught whelks) in Wassaw Sound, Georgia, closely matched my growth curves for heavily ornamented whelks. Power et al. (2009) estimated 100 mm long mature females as being 6 years old, which agrees with my age estimates. As most whelks at Wassaw Sound are heavily ornamented (Chapter 3), a closer agreement with heavily- vs. weakly- ornamented morphs is expected. Walker et al. (2008) observed variation in growth rates both amongst individuals and between localities in Wassaw Sound in a large mark-recapture study; differences in growth curves may reflect either individual level variation in growth rate or differences throughout the sound.

Kraeuter et al.'s (1989) study, which reared whelks in the laboratory and constructed an average growth curve, matched my curves for weakly ornamented whelks from New York, particularly early in ontogeny. As Kraeuter et al.'s study was conducted in Virginia, it would have exclusively used weakly ornamented whelks, as no other morphologies are present north of Cape Hatteras (Chapter 3). Deviations between my growth curves and Kraeuter et al.'s rates later in ontogeny likely reflect the inclusion of male whelks in their sample, as well as differences in growth rates occurring under laboratory vs. natural conditions. The confounding effect of including both genders (their study incorrectly identified whelks as protandric

hermaphrodites, which makes it both difficult to assess the composition of their sample, and suggests that it was mixed) could produce the observed pattern of lower growth rates later in ontogeny. Power et al. (2009) found substantial differences in growth rate between males and female whelks in Georgia, as male whelks, after a period of initially rapid growth, decreased growth rates after reaching reproductive maturity at age 4; Walker et al. (2008) found differences in size distributions between genders, with males overall smaller. While the breakdown of genders used in Kraeuter et al.'s study cannot be determined, both individual variation in growth rates as well as the effects of including males within the sample likely contributed to the observed discrepancy between rates.

Agreement between my growth curves and growth models obtained in Eversole et al.'s 2008 South Carolina study was poor: however, this is likely an artifact of very different treatment of data. Eversole et al. (2008) excluded incidences of negative and non-growth from the sample used to construct their growth model, though they noted in their study that negative growth in some samples approached 50%. As *Busycon carica* grows episodically, the exclusion of incidences of zero growth (or negative growth) unnecessarily excludes a large amount of biologically relevant data. Bruce et al.'s sample was also skewed towards larger, mature individuals, which would lead to lower estimates of growth rates overall.

Stable Isotope Analysis

Oxygen isotope values for LIN I and LIN II were very negative (shifted approximately 1.5 ‰ from the Georgia specimens), which suggests a strong freshwater influence (e.g., Fairbanks, 1982; Geary et al., 1992; Ivany et al., 2003), likely due to isotopically depleted groundwater input into Peconic Bay (LaRoche et al., 1997). Carbon isotopes can be influenced by a number of factors, including upwelling, freshwater runoff, temperature, and vital effects

occurring during fractionation (e.g., Geary et al., 1992). Carbon has been shown in some mollusks to vary annually, which may result from annual variation in any of the above factors (Krantz, 1990; Geary et al., 1992. As mentioned in Results, while carbon does seem to cycle yearly, profiles for Wassaw Sound whelks do not correlate well with temperature, and do not seem to systematically either lag or precede peaks in the oxygen isotope curve. In Wassaw Sound, both productivity and precipitation vary annually; either of these factors may explain the apparent annual signal in the carbon curve (Verity et al., 1993; Verity & Borkman, 2010). In contrast, carbon isotopes for Long Island specimens are weakly but positively correlated with temperature, and are much more positive (1.5-2.0‰) than Wassaw Sound samples. Values are likely related to heavy groundwater input into Peconic Bay, as freshwater tends to have a positive carbon signature (Geary et al., 1992).

Large shifts in oxygen and carbon values from sample to sample later in growth profiles for Wassaw Sound whelks may result from increasingly episodic addition of new growth increments in older individuals. Later in ontogeny, Long Island whelks appear to add more intervals to their shells annually as compared to Wassaw Sound whelks, which results in lower amplitude variation from sample to sample. While variation within possible growth intervals was observed for LIN I, heavy wear on the external surface of the shell made it difficult to determine if this was a result of growth intervals being added over a longer time period, or incorrect interpretation of where boundaries between growth intervals occurred.

Adaptive Trade-Offs

As mentioned above, the two morphologies present within Wassaw Sound display markedly different growth rates. Within mollusks, predation is frequently implicated as driving the evolution of structural defenses (e.g. Palmer, 1979; Vermeij, 1987; Vermeij, 1993; Trussell

& Smith, 2000; Trussell & Nicklin, 2002; Delgado et al., 2002), and the evolution of a well-ornamented morphology in the southern province may be in response to heightened predation pressure at lower latitudes (e.g., Pianka, 1966; Vermeij, 1987; Vermeij, 1993; Coley & Barone, 1996; Schemske et al., 2009; Freestone et al., 2011). This suggests that adaptive trade-offs are likely associated with both morphologies. Well-ornamented individuals are more likely to survive predatory attacks (Chapter 3); however, larger individuals are generally more fecund than smaller individuals within ectotherms (Peters, 1983). Therefore, the ability of weakly-ornamented individuals to rapidly attain large body sizes should increase their reproductive output, even if they are more vulnerable to predation at small size classes. Additionally, in similarly sized individuals from other gastropod species, well-ornamented morphologies produce less offspring than weakly ornamented individuals, as resources are allocated away from reproduction towards the maintainance of shell armor (Geller, 1990). Reproductive trade-offs likely allow the weakly ornamented morphologies to co-exist with the less-vulnerable well-ornamented morphologies in high-predation environments, even though their increased predation vulnerability makes it less likely that weakly-ornamented individuals will reach maturity. Future large mark-recapture studies should consider morphology in population level growth estimates.

Growth and seasonality

Egg cases are laid by *B. carica* in Virginia in the fall (Castagna & Kraeuter, 1994), whereas in North Carolina and Georgia egg cases are laid in both the fall and spring (Magalhaes, 1948; Walker, 1988; Power et al., 2002) and hatch six weeks to 13 months after deposition (Edwards & Harasewych, 1988; Power et al., 2002). The Wassaw Sound specimens are interpreted as having hatched in the spring on the basis of initially moderate oxygen isotope values (values over the first 10 to 20 specimens tend to range between -1.00 to -1.5‰) and may

have been laid in the early spring or fall; Long Island specimens are interpreted as having been laid in the fall (e.g., Castagna & Kraeuter, 1994), and then hatched the following spring (initial values around -1.50 to -2.00‰).

Salinity values in Long Island Sound are influenced by freshwater runoff from nearby rivers in addition to groundwater input, with highest freshwater input in the spring and lowest freshwater input (and therefore highest salinity) in the fall (Lee & Lwiza, 2005). Annual variation in salinity is minor, though (~1‰), and should not substantially effect temperature calculations. Estimates of temperature in Wassaw Sound may be more substantially altered by abiotic factors. Precipitation is isotopically light, and water left behind after periods of evaporation in the sound is isotopically heavy. Variation in isotopic composition due to seasonal changes in precipitation and runoff undoubtedly influence oxygen isotope records and therefore temperature estimates. Temperature estimates (calculated from shell isotopes) areoften lower than measured monthly temperatures at this locality. While abiotic effects likely affect temperature estimates, correcting for a range of salinity values still gives a lower maximum temperature at which shell growth occurs than that observed to the north by about 2°C. Presumably, whelks to the south are adapted to grow at temperatures as high as whelks to the north; however, accretion only occurs over a narrow range of temperatures.

Growth in *B. carica* is highly seasonal, a pattern which occurs in several other mollusks (e.g., Ivany et al., 2003; Schone et al., 2007; Schone, 2008, etc): in Wassaw Sound, most shell material is added in November, December, March, and April. In contrast, Long Island whelks add shell material starting in the spring and continuing through the summer to the fall. Kraeuter et al. (1989) observed cessation of growth in northern province whelks from Virginia from early November to late March, which matches observations for LIN I and LIN II. In Georgia, whelks

bury subtidally at 12°C, but actively seek out food at water temperatures above 14°C (Walker et al., 2008). The narrow window of temperatures over which whelks add to their shells in the southern province may be related to their feeding ecology, in which snails chip open prey items using the lips of their own shells (e.g., Colton, 1908; Warren, 1916; Magalhaes, 1948; Carriker, 1951; Paine, 1962; Kent, 1983; Dietl, 2003a; Dietl, 2003b). Shell breakage can occur during feeding, and diminishes the ability of whelks to subjugate future prey items until they repair their shells (Dietl, 2003). As whelks in Georgia actively forage in the spring and fall (Walker, 1988; Walker et al., 2004; Walker et al., 2008; Shalack et al., 2011), this truncated growth period may reflect a balance in time spent feeding vs. growing. Growth conditions to the north are predicted to be less hospitable, as calcium carbonate availability is decreased in cooler waters (Vermeij, 1993); however, the longer effective growing season appears to compensate for harsher abiotic conditions, leading to only a slightly lower growth rate when compared to weakly ornamented southern individuals.

Episodic Growth

Growth in busyconine whelks is highly episodic, and becomes increasingly more so throughout ontogeny. This presents a unique challenge when interpreting isotopic profiles. While the range of temperatures over which whelks added to their shells did not change throughout ontogeny, the number of new shell intervals added per year decreased. Heavily ornamented individuals (WSS II and WSS III) illustrate well the challenges of interpreting growth rates for episodic organisms: the isotope curves of both individuals were very difficult to interpret, as a result of very narrow temperature ranges over which shell material was added for parts of their profiles. Future studies should investigate whether or not this may be related to ecological factors (time spent feeding, shifts in reproduction).

Conservation Applications

Current minimum allowable lengths in the northern province (Delaware, 127 mm; Maryland, 152 mm; Massachusetts, 70 mm shell width; New Jersey, 127 mm) allow the harvest of pre-reproductive individuals: by the age estimates presented here, individuals may be removed from fisheries at 6 or 7 years of age, well before they begin reproducing (Castagna & Kraeuter, 1994). In the southern province, only one state currently has a minimum size limit (South Carolina, 102 mm [Bruce, 2006]). While individuals mature at a younger age to the south, the absence of a size limit in Georgia and North Carolina in combination with the low size limit in South Carolina may lead to rapid depletion of stocks.

The present study suggests that harvest size limits to the north should be increased, and that harvest limits to the south should consider additionally the morphology of specimens. Further studies should investigate age at maturity for the two different morphologies present to the south as well as differences in fecundity, which may affect this species' vulnerability to both overfishing pressure and environmental changes. As harvests of *Busycon* increase, a better understanding of the species' ecology could help ensure better management and the continued viability of this resource.

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REFERENCES

Abbott, R. T. 1974. American Seashells. 2nd Edition. Van Nostrand Reinhold Co., New York. 663 p.

Allmon, W.D., Jones, D.S. & N. Vaughan. 1992. Observations on the biology of *Turritella gonostoma Valenciennes* (Prosobranchia: Turritellidae) from the Gulf of California. The Veliger 35(1): 52-63.

Allmon, W.D., Jones, D.S., Aiello, R.L., Gowlett-Holmes, K. & P. K. Probert. 1994. Observations on the biology of *Maoricolpus roseus* (Quoy & Gaimard) (Prosobranchia: Turritellidae) from New Zealand and Tasmania. The Veliger 37 (3):267-279.

Angilletta, M.J., Niewiarowski, P.H., Dunham, A.E., Leache, A.D. & W.P. Porter. 2004. Bergmann's clines in ectotherms: illustrating a life-history perspective with Sceloporine lizards. The American Naturalist 164 (6) e-article.

Ansell, A.D. 1968. The rate of growth of the hard clam *Mercenaria mercenaria* (L) throughout the geographical range. J. Cons. Perm. Int. Explor. Mer. 31 (3):364-409.

Belk, M.C. & D.D. Houston. 2002. Bergmann's Rule in ectotherms: a test using freshwater fishes. The American Naturalist 160 (6):803-808.

Bruce, D.G. 2006. The whelk dredge fishery of Delaware. Journal of Shellfish Research 25 (1): 1-13.

Bruno, S.F., Staker, R.D., & G. M. Sharma. 1980. Dynamics of phytoplankton productivity in the Peconic Bay Estuary, Long Island. Estuarine and Coastal Marine Science 10:247-263.

Buick, D.P. & L.C. Ivany. 2004. 100 years in the dark: extreme longevity of Eocene bivalves from Antarctica. Geology 32(10):921-924.

Calder, D.R. 1992. Similarity analysis of hydroid assemblages along a latitudinal gradient in the western North Atlantic. Can. J. Zool. 70(6):1078-1085.

Carriker, M.R. 1951. Observations on the penetration of tightly closing bivalves by *Busycon* and other predators. Ecology 32(1):73-83.

Castagna, M. & J.N. Kraeuter. 1994. Age, growth rate, sexual dimorphism and fecundity of knobbed whelk *Busycon carica* (Gmelin, 1791) in a western mid-Atlantic lagoon system, Virginia. Journal of Shellfish Research 12:581-585.

Ceci, L. 1980. The first fiscal crisis in New York. Economic development and cultural change 28(4):839-847.

Cledon, M., Brey, T., Penchaszadeh, P.E. & W. Arntz. 2005. Individual growth and somatic production in *Adelomelon brasiliana* (Gastropoda; Volutidae) off Argentina. Marine Biology 147: 447-452.

Coley, P.D. & J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27: 305-335.

Colton, H.S. 1908. How *Fulgur* and *Sycotypus* eat oysters, mussels and clams. Proc. Acad. Nat. Sci. Philadelphia 60: 3–10.

Cook, R.R. & P.J. Auster. 2007. A bioregional classification for the continental shelf of northeastern North America for conservation analysis and planning based on representation. Marine Sanctuaries Conservation Series NMSP-07-03. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Silver Spring, MD. 14 pp.

Davis, J.P. & R.T. Sisson. 1988. Aspects of the biology relating to the fisheries management of New England populations of the whelks, *Busycotypus canaliculatus* and *Busycon carica*. Journal of Shellfish Research 7(3):453-460.

Delgado, G. A., Glazer, R. A., & N.J. Stewart. 2002. Predator-Induced Behavioral and Morphological Plasticity in the Tropical Marine Gastropod *Strombus gigas*. Biological Bulletins 203:112-120.

DeWitt, T.J., Robinson, B.W. & D.S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research 2:129-148.

DiCosimo, J. & W.D. DuPaul. 1985. Preliminary observations of the *Busycon* whelk fishery in Virginia. Journal of Shelfish Research 5:34.

Dietl, G.P. 2003a. Coevolution of a marine gastropod predator and its dangerous bivalve prey. Biological Journal of the Linnean Society 80:409-436.

Dietl, G. P. 2003b. Interaction strength between a predator and dangerous prey : *Sinistrofulgur* predation on *Mercenaria*. Journal of Experimental Marine Biology and Ecology289:287 - 301. doi:10.1016/S0022-0981(03)00047-9

Edwards, A.L. 1988. Latitudinal clines in shell morphologies of *Busycon carica* (Gmelin 1791). Journal of Shellfish Research 7(3):461-466.

Edwards, A.L. & C.M. Humphrey. 1981. An electrophoretic and morphological survey of *Busycon* occurring in Wassaw Sound, Georgia. The Nautilus 95(3):144-150.

Edwards, A.L. & M.G. Harasewych. 1988. Biology of the recent species of the subfamily Busyconinae. Journal of Shellfish Research 7(3):467-472.

Engle, V.D. & J.K. Summers. 1999. Latitudinal gradients in benthic community composition in Western Atlantic estuaries. Journal of Biogeography 26:1007-1023.

Epstein, S., Buchsbaum, R., Lowenstam, H. A., & H.C. Urey. 1953. Revised carbonate-water isotopic temperature scale. Geol. Soc. Am. Bull 64:1315-1325.

Eversole, A.G., Anderwon, W.D., & J.J. Isely. 2008. Age and growth of the knobbed whelk *Busycon carica* (Gmelin 1791) in South Carolina subtidal waters. Journal of Shellfish Research 27(2):423-426.

Fairbanks, R.G. 1982. The origin of continental shelf and slope water in the New York Bite and gulf of Maine-evidence from $H_2{}^{18}O/{}^{16}O$ ratio measurements: Journal of Geophysical Research 87:5796-5808.

Frank, P.W. 1975. Latitudinal variation in the life history features of the black turban snail *Tegula funebralis* (Prosobranchia: Trochidae). Marine Biology 31:181-192.

Freestone, A.L., Osman, R.W., Ruiz, G.M.,& M. E. Torchin. 2011. Stronger predation in the tropics shapes species richness patterns in marine communities. Ecology 92:983–993. [doi:10.1890/09-2379.1]

Geary, D.H., Brieske, T.A., & B.E. Bemis. 1992. The influence and interaction of temperature, salinity, and upwelling on the stable isotopic profiles of strombid gastropod shells. Palaios 7(1):77-85.

Geller, J.B. 1990. Consequences of a morphological defense: growth, repair and reproduction by thin-and thick-shelled morphs of *Nucella emarginata* (Deshayes)(Gastropoda:Prosobranchia). J. Exp. Mar. Biol. Ecol. 144:173-184.

Gentry, D.K., Sosdian, Sindia, Grossman, E.L., Rosenthal, Y., Hicks, D. & C.H. Lear. 2008. Stable isotope and Sr/Ca profiles from the marine gastropod *Conus ermineus*: testing a multiproxy approach for inferring paleotemperature and paleosalinity.Palaios 23:195-209.

Goodwin, D.H., Flessa, K.W., Schone, B.R., & D.L. Dettman. 2001. Cross-calibration of daily growth increments, stable isotope variation, and temperature in the Gulf of California bivalve mollusk *Chione cortezi*: implications for paleoenvironmental analysis. Palaios (16):387-398.

Goodwin, D.H., Schone, B.R., & D. L. Dettman. 2003. Resolution and fidelity of oxygen isotopes as paleotemperature proxies in bivalve mollusk shells: models and observations. Palaios 18 (2)110-125.

Grossman, E.L. & T. Ku. 1986. Oxygen and carbon isotope fractionation in biogenic aragonite: Temperature effects. Chemical Geology 59:59-74.

Hall, C.A. 1964. Shallow-water marine climates and molluscan provinces. Ecology 45(2):226-234.

Hayden, B.P. & R. Dolan. 1976. Coastal marine fauna and marine climates of the Americas. Journal of Biogeography 3(1):71-81.

Hayden, B.P., Ray, G.C. & R. Dolan. 1984. Classification of coastal and marine environments. Environmental Conservation 11(3):199-207.

Heibo, E., Magnhagen, C., & L. A. Vollestad. 2005. Latitudinal variation in life-history traits in Eurasian perch. Ecology 86(12):3377-3386.

Henry, K.M. & R.M. Cerrato. 2007. The annual macroscopic growth pattern of the northern quahog (hard clam, *Mercenaria mercenaria*[Linne]), in Narragansett Bay, Rhode Island. Journal of Shellfish Research 26 (4):985-993.

Hollister, S. C. 1958. A review of the genus *Busycon* and its allies. Paleontog. Amer., 4: 49-126.

Hoso, M. & M. Hori. 2008. Divergent shell shape as an antipredator adaptation in tropical land snails. The American Naturalist 172(5):726-732.

Ivany, L.C., Wilkinson, B.H., & D.S. Jones. 2003. Using stable isotopic data to resolve rate and duration of growth throughout ontogeny: an example from the surf clam, *Spisula solidissima*. Palaios 18:126-137.

Jones, D.S., Williams, D.F. & C.S. Romanek. 1986. Life history of symbiont-bearing giant clams from stable isotope profiles. Science 231(4733):46-48.

Jones, D.S. 1988. Isotopic determination of growth and longevity in fossil and modern invertebrates. In Paleontological Society Papers 4, Isotope Paleobiology and paleoecology. Eds. Norris & Colefield. Pp. 37-67.

Jones, D.S., Arthur, M.A., & D. J. Allard. 1989. Sclerochronological records of temperature and growth from shells of *Mercenaria mercenaria* from Narragansett Bay, Rhode Island. Marine Biology 102, 225-234.

Jones, D.S. & I.R. Quitmyer. 1996. Marking time with bivalve shells: oxygen isotopes and season of annual increment formation. Palaios 11 (4):340-346.

Kent, B.W., 1983. Patterns of coexistence in Busyconine whelks. J. Exp. Mar. Biol. Ecol. 66: 257–283.

Kosloski, M.E. & G.P. Dietl. 2011. The use of modern death assemblages to test the geographic mosaic theory of coevolution. Geological Society of America Abstracts with Programs, Vol. 43, No. 5, p. 504

Kraeuter, J.N., Castagna, M., Bisker, R. 1989. Growth rate estimates for *Busycon carica* (Gmelin, 1791) in Virginia. Journal of Shellfish Research 8(1):219-225.

Krantz, D.E. 1990. Mollusk-Isotope Records of Plio-Pleistocene Marine Paleoclimate, U.S. Middle Atlantic Coastal Plain. Palaios 5(4):317-335.

Krantz, D.E., Jones, D.S., & D.F. Williams. 1984. Growth rates of the sea scallop, *Plagopecten magellanicus*, determined from the 18 O/16 O record in shell calcite. Biological Bulletin 167(1):186-199.

Krantz, D.E., Williams, D.F., & D.S. Jones. 1987. Ecological and paleoenvironmental information using stable isotope profiles from living and fossil molluscs. Palaeogeography, Palaeoclimatology, Palaeoecology 58:249-266.

LaRoche, J., Nuzzi, R., Waters, R., Wyman, K., Falkowski, P.G., Wallace, D.W.R. 1997. Brown tide blooms in Long Island's coastal waters linked to interannual variability in groundwater flow. Global Change Biology 3:397-410.

Lee, H.J., & E.G. Boulding. 2010. Latitudinal clines in body size, but not in thermal tolerance or heat-shock cognate 70 (HSC70), in the highly-dispersing intertidal gastropod *Littorina keenae* (Gastropoda: Littorinidae). Biological Journal of the Linnean Society 100: 494-505.

Lee, Y.J. & K. Lwiza. 2005. Interannual variability of temperature and salinity in shallow water: Long Island Sound, New York. Journal of Geophysical Research 110: C09022.

Leiva, G.E. & J.C. Castilla. 2002. A review of the world marine gastropod fishery: evolution of catches, management, and the Chilean experience. Reviews in Fish Biology and Fisheries 11:283-300.

Magalhaes, H. 1948. An ecological study of snails of the genus *Busycon* at Beaufort, North Carolina. Ecological Monographs 18 (3)377-409.

Moore, C.B. 1921. Notes on shell implements from Florida. American Anthropologist 23(1):12-18.

Munch, S.B. & S. Salinas. 2009. Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. PNAS 106(33):13860-13864.

Olabarria, C., & M.H. Thurston. 2003. Latitudinal and bathymetric trends in body size of the deep-sea gastropod *Troschelia berniciensis* (King). Marine Biology 143: 723-730.

Paine, R.T., 1962. Ecological diversification in sympatric gastropods of the genus *Busycon*. Evolution 16: 515–523.

Palmer, A.R. 1979. Fish predation and the evolution of gastropod shell sculpture: experimental and geographic evidence. Ecology 33:697-713.

Palmer, A.R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). Hydrobiologia 193 (1) 155-182.

Parsons, K.E. 1997. Contrasting Patterns of Heritable Geographic Variation in Shell Morphology and Growth Potential in the Marine Gastropod *Bembicium vittatum*: Evidence from Field Experiments. Evolution 51(3):784-796.

Paul, A.J. & H.M. Feder. 1973. Growth, recruitment, and distribution of the littleneck clam, *Protothaca staminea*, in Galena Bay, Prince William Sound, Alaska. Fishery Bulletin 71:665-677.

"Peconic Bay, Long Island". Map. *Google Maps*. Google, 15 May 2012. Web, 15 May 2012.

Peters, R.H. 1983. The ecological impacts of body size. Cambridge University Press, Cambridge.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: a review of concepts. American Naturalist 100:33-46.

Posilovic, H. & Z. Bajraktarevic. 2010. Functional morphological analysis of evolution of ribbing in Pliocene viviparid shells from Croatia. Lethaia 43:457-464.

Power, A.J., Covington, E., Recicar, T., Walker, R.L., & N. Eller. 2002. Observations on the egg capsules and hatchlings of the knobbed whelk, *Busycon carica* Gmelin 1791) in coastal Georgia. Journal of Shellfish Research 21(2):769-775.

Power, A.J., Sellers, C.J., & R.L. Walker. 2009. Growth and sexual maturity of the knobbed whelk, *Busycon carica* (Gmelin 1791), from a commercially harvested population in coastal Georgia. Occasional papers of the University of Georgia Marine Extension Service, Vol. 4, 24 pp.

Quitmyer, I. R. & Reitz, E. J. 2006. Marine trophic levels targeted between AD 300 and 1500 on the Georgia coast, USA. Journal of Archaeological Science 33: 806-822.

Richardson, C.A., Saurel, C., Barroso, C.M., Thain, J. 2005. Evaluation of the age of the red whelk *Neptunea antiqua* using statoliths, opercula and element ratios in the shell. Journal of Experimental Marine Biology and Ecology 325:55-64.

Schemske, D. W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M., & K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions? Annual Review of Ecology, Evolution and Systematics 40: 245-269.

Schone, B.R. 2008. The curse of physiology-challenges and opportunities in the interpretation of geochemical data from mollusk shells. Geo-Mar Lett 28:269-285.

Schone, B.R., Houk, S.D., Freyre Castro, A. D., Fiebig, J., Oschmann, W., Kroncke, I., Dreyer, W. & F. Gosselck. 2005. Daily growth rates in shells of *Acrtica islandica*: assessing sub-seasonal environmental controls on a long-lived bivalve mollusk. Palaios 20 (1):78-92.

Schone, B.R., Rodland, D.L., Wehrmann, A., Heidel, B., Oschmann, W., Zhang, Z., Fiebig, J. & L. Beck. 2007. Combined sclerochronologic and oxygen isotope analysis of gastropod shells (*Gibbula cineraria*, North Sea): life-history traits and utility as a high-resolution environmental archive for kelp forests. Marine Biology 150:1237-1252.

Shalack, J.D., Power, A.J., & R.L. Walker. 2011. Hand harvesting quickly depletes intertidal whelk populations. American Malacological Bulletin 29: 37-50.

Trussell, G.C. & R.J. Etter. 2001. Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. Genetica 112-113:321-337.

Trussell, G.C. & L.D. Smith. 2000. Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. PNAS 97(5) 2123-2127.

Trussell, G.C. & M.O. Nicklin. 2002. Cue sensitivity, Inducible defense, and trade-offs in a marine snail. Ecology 83 (6)1635-1647.

Verity, P.G., Yoder, J.A., Bishop, S.S., Nelson, J.R., Craven, D.B., Blanton, J.O., Robertson, C.Y. & C.R. Tronzos. 1993. Composition, productivity and nutrient chemistry of a coastal ocean planktonic food web. Continental Shelf Research 13(7):741-776.

Verity, P.G. & D.G. Borkman. 2010. A decade of change in the Skidaway river estuary. III. Plankton. Estuaries and Coasts 33:513-540.

Vermeij, G.J. 1987. Evolution and Escalation. An Ecological History of Life. Princeton University Press, Princeton, N. J. 527 pp

Vermeij, G.J. 1993. A Natural History of Shells. Princeton University Press, Princeton, NJ. 207 pp.

Walker, R.L. 1988. Observations on intertidal whelk (*Busycon* and *Busycotypus*) populations in Wassaw Sound, Georgia. Journal of Shellfish Research 7 (3):473-478.

Walker, R.L., Smith, J.H. & A.J. Power. 2004. Movement and behavioral patterns of whelks on intertidal flats in Wassaw Sound, Georgia. Marine Extension Bulletin No. 29, October 2004. 18 pp.

Walker, R.L., Power, A.J., Sweeney-Reeves, M., Covingeton, E., Mithcell, M. & T. Recicar. 2008. Growth, migration, population structure and sex ratio of four whelk species (Family Melogenidae) within Wassaw Sound, Georgia. Occasional papers of the University of Georgia Marine Extension Service, Vol. 1., 2008. 46 pp.

Warren, S., 1916. The feeding habits of *Busycon*. Nautilus 30: 66–68.

"Wassaw Sound, Georgia". Map. *Google Maps*. Google, 15 May 2012. Web, 15 May 2012.

Wilber, K.M. & A.S.M. Saleuddin. 1983. Shell formation. Pages 236-287 in K.M. Wilber, ed. The Mollusca: Vol. 4, Physiology, Part 1. New York Academic Press, New York. 523 p.

CHAPTER 3

ANATOMY OF A CLINE: DISSECTING ANTI-PREDATORY ADAPTATIONS IN A MARINE GASTROPOD ALONG THE U.S. ATLANTIC COAST

ABSTRACT

Adaptation to local ecological conditions is widely posited to shape intraspecific geographic variation in morphology. This study characterizes anti-predatory adaptive morphology within a single marine gastropod species (the knobbed whelk, *Busycon carica*) over a large (>1,000 km) geographic area. I used repair scar data to estimate predation frequencies in wild populations, and experimental predation studies with the powerful durophagous predator, the stone crab (*Menippe mercenaria*), to test the effectiveness of several presumed antipredatory morphological traits: the tumid ridge (an elevated ridge that runs along the outside of the body whorl), spines, and increased shell thickness. I used linear morphometrics to characterize shell size and thickness, spinosity, and the distribution of the tumid ridge throughout *B. carica*'s range. Thick shells were 33% more effective than thinner shells in preventing mortality in laboratory studies. Southern individuals possessed fewer but significantly larger spines, a 13% thicker shell (measured at the shoulder), and a 34% thicker shell (measured along the canal), which is attributed to the development of a pronounced tumid ridge, a feature that does not occur in northern conspecifics. Repair scar frequencies peaked to the south for almost all size classes, and standardization by shell size and morphology strengthened this trend. This study supports the hypothesis that higher predation intensity at lower latitudes drives the evolution of anti-predatory defenses.

INTRODUCTION

The capacity to adapt to local ecological conditions is essential for the survival of individual populations. Such adaptations may include the development or modification of morphological traits, biochemical adjustments, the evolution of new behaviors, and a myriad of other traits. Understanding different species' capacities to respond to environmental change is becoming increasingly important, as human activities continue to accelerate both the rate and magnitude of biotic and abiotic changes (Jackson et al., 2001; Scavia et al., 2002; Halpern et al., 2007; Dietl, 2009).

Local adaptation should be most prevalent in species with relatively large geographic ranges and direct developing larvae, because different populations are exposed to variation in temperature, food, competitors, and/or predation pressure (Cody, 1966; Vermeij, 1978; Bertness et al., 1981). There are a number of well worked terrestrial studies of long-ranging species (ranges over 100's of km) that provide evidence of widespread local adaptation, resulting in large-scale clines in morphological and physiological traits. For instance, Laurila et al. (2008) examined differences in anti-predatory defenses in the frog, *Rana temporaria*, over a 1500 km transect across Sweden and found a suite of life history traits that correlated with higher predator frequencies at southern localities. Similarly, Toju and Sota (2006) examined co-evolution between the Japanese camellia (*Camellia japonica*) and the camellia weevil (*Curculio camelliae*) and found correlations between pericarp thickness and the size of the weevil's defensive apparatus across a 700 km distance. Many well-worked marine examples examine adaptive responses to abiotic selective pressures. Mussels (*Mytilus edulis*) show clines in shell color that may be related to different thermoregulatory demands at high and low latitudes along the east

coast of North America (Mitton, 1977). In a number of systems, marine mollusks exhibit latitudinal clines in body size and/or growth rates, which are hypothesized to be an adaptation to clinal variation in mean annual temperature (e.g., Frank, 1975; Olabarria & Thurston, 2003; Munch & Salinas, 2009; Lee & Boulding, 2010). Significant morphological evolution has also occured in at least one system (the marine gastropod *Acanthinucella spirata*) during post-Pleistocene range expansion, though the precise selective factors controlling adaptation for *Acanthinucella* are not well constrained (Hellberg et al., 2001).

Marine examples of responses to biotic pressures, however, are less common. One of the best studies of long-ranging local adaptation in a marine invertebrate predator-prey system is Stachowicz and Hay's (2000) work on the decorator crab, *Libinia dubia*. Decorator crabs show a cline in usage of toxic algae as shell camouflage along approximately 1,000 km of the eastern Atlantic Coast of the United States: preferential use of toxic algae is correlated with elevated predation pressures in the southern part of the species range. Within gastropods, the marine snail *Littorina obtusata* shows a modern morphological cline mediated by different expression of phenotypically plastic shell features, with thicker shells to the south. The cline is thought to be shaped by a combination of biotic (predation) and abiotic (temperature variation) factors (Trussell & Etter, 2001; Trussell & Nicklin, 2002). *Littorina obtusata* also shows a temporal increase in shell thickness from the mid 1800s onward, which is postulated to be in response to the introduction and spread of the predatory green crab, *Carcinus maenas* (Seeley, 1986; Trussell & Smith, 2000). Work on this system suggests that adaptive responses to predatory regimes can evolve very rapidly (at least, when plastic responses are involved) over large spatial scales. Fitness trade-offs between shell thickness and body mass as well as linear growth rates may additionally control the overall distribution of adaptations within this species (Trussell & Smith,

2000; Trussell & Nicklin, 2002). Anthropogenic forces may also impose selective pressures within populations, and have been implicated in driving size declines in rocky intertidal gastropods from California (Roy et al., 2003).

This study examines local adaptation to predators in a marine gastropod, *Busycon carica*, which occurs over a large geographic range (ten degrees of latitude, or approximately 1,100 km) along the eastern coast of the U.S. This range encompasses two distinctive biogeographic provinces: the warm-temperate Carolinian Province, which extends from Cape Hatteras, North Carolina to Cape Canaveral, Florida, and the cooler Virginian Province, which extends from Cape Hatteras northwards to Cape Cod, Massachusetts (Engle & Summers, 1999). Abundant evidence suggests that biotic interactions are strongest at lower latitudes (Pianka, 1966; Vermeij, 1987; Coley & Barone, 1996; Schemske et al., 2009; Freestone et al., 2011); therefore, we predict that the expression of anti-predatory adaptations in the knobbed whelk's shell should be greatest in the southern part of its range, where predation pressure is highest.

Study System

The knobbed whelk, *Busycon carica* (Family Melongenidae, subfamily Busyconinae) is a large, long-lived, predatory gastropod, which feeds on bivalve prey by wedging the valves of its prey open with its shell lip (Colton, 1908; Warren, 1916; Magalhaes, 1948; Carriker, 1951; Paine, 1962; Kent, 1983; Dietl, 2003a; Dietl, 2003b). It is relatively mobile (one mark-recapture study showed average movement of 18 meters/day [Magalhaes, 1948]), and common in intertidal and near-shore environments. The shell of *B. carica* is also highly variable throughout its range (Edwards, 1988), with a "northern" morphology characterized by a high spire and small spines in contrast to a "southern" morphology, with longer spines, a more massive shell, a lower spire, and a pronounced swelling that wraps around the body whorl, known as the "tumid ridge" (Hollister,

1958; Abbott, 1974; Edwards, 1988) (Figure 3.1). Both the northern and southern morphologies are present throughout the southern part of *B. carica*'s range, though the relative frequencies of the two vary within different populations. This high level of morphological variation and its latitudinal partitioning have led several authors to split *Busycon* populations taxonomically at both the species and subspecies level (Abbott, 1954; Hollister, 1958; Petuch, 1994), although genetic studies as well as several quantitative morphological studies have shown a high degree of intergradation between the two morphologies and suggested that splitting at or below the species level is not justified (Abbott, 1974; Edwards & Humphrey, 1981; Edwards & Harasewych, 1988; Berlocher, 2000).

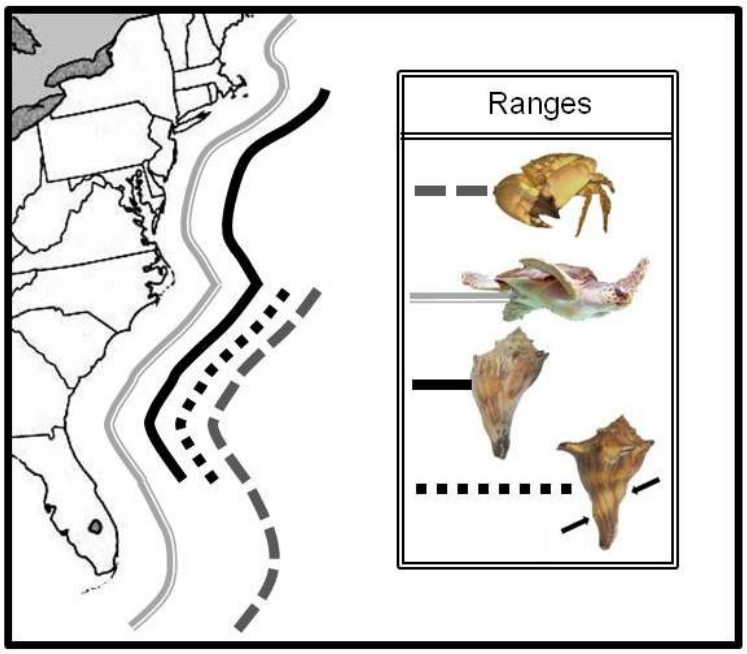

Figure 3.1. Geographic ranges of the knobbed whelk *Busycon carica* and two durophagous predators, the stone crab *Menippe mercenaria* and the loggerhead turtle *Caretta caretta* along the eastern coast of the United States. Whelk morphology with largest distribution lacks tumid ridge, and possesses numerous small spines. Whelks inhabiting southern part of species range may show well-developed tumid ridge and large spines (arrows). Photograph of Loggerhead Turtle by Mike Gonzalez via Wikimedia Commons; taken at the Georgia Aquarium, Atlanta, Georgia, 14 October 2007.

The shell of *B. carica* shows variation in a number of presumed ecologically important anti-predatory traits. *Large body size* may provide a refuge from predation, allowing predator and prey species to co-exist in close proximity (Paine, 1976). A size refuge may result from the mechanics of predation: 1.) many predators are not capable of handling prey above a maximum length (Paine, 1976); and 2.) predators may avoid prey above a given size due to increasing handling time and costs (Vermeij, 1993). *Spines* are a conspicuous feature on the shoulder of the shell throughout the knobbed whelk's range, and have been shown in other mollusks to serve as effective anti-predatory defenses against a variety of durophagous (shell-crushing) predators by localizing stress at thicker parts of shells, and distributing stress over larger areas (Palmer, 1979). Large spines may also provide defense against gape-limited jawed predators by increasing shell width. Increased *shell thickness* is often associated experimentally with improved defensive performance, and thickened shell walls may be the most reliable defense against crushing predators (Vermeij, 1993; Zuschin et al., 2003). Thickening may be accomplished either by adding shell material evenly over the surface of the shell (i.e., thickening the entire shell wall), or by preferentially adding to specific regions of the shell (i.e., thickening only at one portion along the lip). Producing shell material is energetically expensive (1/3 to 1/4 of the total expenditure for growth [Wilber & Saleuddin, 1983]), and localized shell thickening may confer equivalent increases in defense as compared to more evenly distributed increases in shell thickness. The *tumid ridge* is an example of localized shell thickening.

A host of different durophagous predators (*Caretta caretta*, the loggerhead turtle; various crab species—particularly genus *Menippe*; bony fish and rays) occur throughout the range of *Busycon carica* (Figure 3.1). Species present vary latitudinally in both strength and abundance. *Caretta caretta* is reported in the literature as able to break shells of the large marine gastropod

Strombus gigas up to 15 mm in thickness (Randall, 1964). Loggerhead turtles are cosmopolitan species that have evolved short, strong mandibles as a foraging adaptation for hard-shelled mollusks and crustaceans (Kamezaki, 2003). Diet is mainly inferred from gut contents, with hard-shelled mollusks (including *Busycon* species) making up a large component in the southern United States (Hopkins-Murphy et al., 2003; Wallace et al., 2009). In the United States, loggerheads nest predominantly from North Carolina to Florida (Ehrhart et al., 2003).

Menippe mercenaria (the stone crab) is the largest durophagous crab co-occurring with the knobbed whelk, which ranges along the shallow shelf from Cape Hatteras, NC around Florida to the Gulf of Mexico, where it hybridizes extensively with *Menippe adina* in northwest Florida (Bert, 1986; Bert & Harrison, 1988). *Menippe mercenaria* possesses unusually large claws for a western Atlantic crab, has a high mechanical advantage (Vermeij, 1977; Bert, 1986), and can generate forces up to 2000 kN m^{-2} (Blundon, 1988). *Menippe mercenaria* has been noted as the only western Atlantic brachyuran crab possessing "truly large claws" (Vermeij, 1977). Stone crabs are highly specialized predators that prey on bivalves, gastropods and hermit crabs by shell crushing (Schenck & Wainwright, 2001; Hughes & Grabowski, 2006). This species is known to prey on *B. carica* in the wild, and on occasion whelk shell piles are found outside of crab lairs (Magalhaes, 1948). As with the loggerhead turtle, *Menippe* is one of the few predators capable of crushing the "southern" knobbed whelk morphology present in populations south of Cape Hatteras, NC.

Durophagous predators impose selective pressures on whelk prey in three ways: 1.) increased mortality, 2.) necessitating energetically expensive shell-repairs following unsuccessful attacks, and 3.) forcing prey to make costly behavioral changes (i.e., changes in feeding or activity levels, escape responses, etc.[Appleton & Palmer, 1988; Palmer, 1990;

Behrens Yamada et al., 1998; Dalziel & Boulding, 2005; Brookes & Rochette, 2007]). Selection should favor increased expression of whelk defensive traits—such as large size, thick shells, and large spines— in areas where predation pressure from shell-crushing predators is intense.

METHODS

Predation Experiments

Predation experiments were designed to test whether presumed anti-predatory adaptations (e.g., the tumid ridge, increased shell thickness, the presence of large spines) functioned effectively against durophagous predators. 50 live specimens of *Busycon carica*, ranging in length from 80 to 110 millimeters, were obtained from Cabbage Island in Wassaw Sound, Georgia. These specimens constituted equal numbers of the typically southern (few large spines, well-developed tumid ridge) and typically northern (many small spines, tumid ridge absent) morphologies present at this locality (Figure 3.1). Knobbed whelks were transported to the Paleontological Research Institution in Ithaca, New York, where all experiments were performed, and kept in 37.5 liter aquaria with re-circulated sea water. Five large male stone crabs (carapace widths from 8-10 centimeters, four with the larger, crushing claw on the right side of the body and one with the crushing claw on the left side of the body) were obtained from Gulf Specimen Company in Panacea, Florida. Prior experimental work has shown that predators with the same chirality as their prey are more effective (Inoda et al., 2003; Dietl & Hendricks, 2006; Hoso et al., 2007); however, as a result of modern fisheries practices, many stone crabs in the wild, which typically have the larger crusher claw on the right side of the body, are currently "left-handed" (Simonson, 1985; T. Bert, personal communications 2010). All tanks were kept

between 18-20° C for the duration of the experiment, and salinity was maintained between 28 and 32 parts per thousand. Approximately 10 centimeters of sand covered the bottom of the tanks, and terra cotta shelters (halved flower pots) were available as shelters for the stone crabs. The sides of tanks were blackened to minimize agonistic interactions between adjacent crabs.

Individual crabs were fed specimens of *Busycon carica* weekly for 10-week periods between fall 2009 and early spring 2011, and interactions were recorded using a Nikon D60 camera and Camera Control Pro (Nikon). The morphologies of the whelk specimens offered were randomized in five two-week lots of two, with the first specimen of every two-week trial determined by coin toss. Morphologies were visually assessed, with well-armored specimens representing individuals with a pronounced tumid ridge and long spines, and weakly armored specimens representing individuals lacking a tumid ridge and with smaller spines (Figure 3.1). The specimen offered in the subsequent trial was the opposite morphology. Measurements of length, spinosity (shell width at the shoulder, attributable to spines), and thickness at the location of the tumid ridge and at the shoulder (Figure 3.2) were taken prior to the experiment using digital calipers to the nearest 0.01 mm. One stone crab completed only seven out of 10 experimental trials before dying. Once placed in the tank, the whelks were left for four hours. Interactions were recorded using time-lapse photography, with images taken every 30 seconds for the duration of the experiment. Handling was characterized by the orientations crabs used to hold shells (aperture up or down, spire up or down, canal up or down) and by the region of the shell that crabs attempted to break (i.e., crushing across the spire, crushing across the aperture, crushing across the body whorl, or crushing across the canal). At the end of the four hours, two live individuals of the bivalve *Mercenaria mercenaria* were placed in the tank for the stone crab to consume and the whelk was removed, photographed, and if still alive, placed back into a

holding tank with other survivors. Individual whelks were only used once in experimental trials. Whelks that were killed during the four-hour time period were photographed and any remaining flesh was removed from their shells. Both before and after being used for experiments, whelks were fed live specimens of *Mercenaria mercenaria* weekly to bi-weekly.

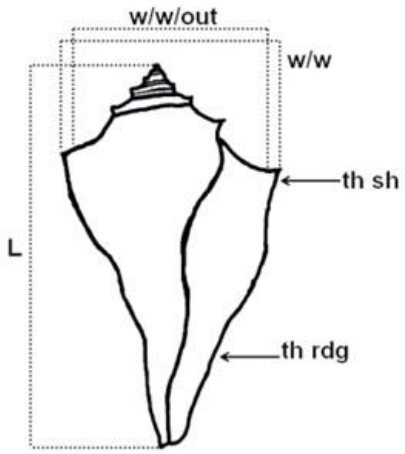

Figure 3.2. Linear measurements. *L* represents shell length, *w/w/out* =width exclusive of spines, *w/w* = shell width inclusive of spines, *th sh* = thickness measured at the shoulder, and *th rdg*= thickness measured at the tumid ridge.

Upon removal from the experiment, whelks were assessed to determine the amount of shell damage, which was scored on a scale of 0 to 5, with 0 representing no obvious damage to the shell and a score of 5 representing fragmentation of the shell into multiple large pieces (Figure 3.3). The relative amount of damage sustained by specimens of each morphology was compared using a Kolmogorov-Smirnov test. Surviving whelks were routinely checked for deteriorating condition. Whelks that died within 2 weeks of the attack were classified as killed by the stone crab, an assumption justified by the observations that: 1.) most whelks that died within this time period had suffered severe tissue damage (Damage Class 4), i.e. removal of the operculum or puncturing and tearing of the soft tissue; and 2.) overall survival rates of whelks in holding tanks, after an initial acclimation period of two weeks, was close to 100%. Survival

frequency was assessed using a two sample Chi- square test. A Mann-Whitney U test was used to evaluate whether differences in observed thickness were statistically significant between the two morphologies used in the experiment. All statistical tests for experimental work were completed in PAST (Hammer et al., 2001).

Damage Class	0	1	2	3	4	5
Description of damage	 Minor or no visible damage to shell	 Minor damage to first growth interval, concentrated along lip	 Extensive damage along lip, may extend to second growth interval	 Damage extends into second or third prior growth intervals, but flesh is not damaged. Canal may be broken off	 Severe damage extending across multiple growth intervals, deeply embayed and/or shell punctured: flesh of whelk is damaged	 Shell completely obliterated; broken into multiple fragments

Figure 3.3. Damage classes used to assess severity of stone crab attacks on knobbed whelks in predation experiments.

Linear Morphometrics

Museum- and field-collected samples from 15 localities along the eastern coast of the U.S. were examined to test the prediction that anti-predatory morphologies should be most prevalent in the southern part of *Busycon*'s range. Museum samples were obtained from collections at the Delaware Museum of Natural History, National Museum of Natural History (Smithsonian Institution), the Paleontological Research Institution, and the personal collections of G. Dietl and M. Kosloski. These samples provided coverage throughout most of *Busycon carica*'s range along the Eastern Coast of the U.S. Populations were grouped in two ways: by

locality, with 15 separate localities from 9 states, and by region, with "southern" and "northern" groups reflecting known biogeographic provinces. The southern province encompasses all populations occurring south of Cape Hatteras, North Carolina, and the northern province encompasses populations occurring north of Cape Hatteras. Several measurements (length, number of spines, width both with and without spines, thickness at the shoulder and tumid ridge) were taken using digital calipers to the nearest 0.01 mm to describe the development of armor for individuals along the cline (Figure 3.2).

Size distributions were determined by averaging both the mean length of all specimens over 100 mm within groupings, and by calculating average length of the five largest specimens. Differences in length between provinces were assessed using a Mann Whitney U test, and differences among populations were evaluated using a Kruskal-Wallis test. The tumid ridge was classified visually as present or absent. Thickness at the tumid ridge and at the shoulder for an individual of 150 mm shell length was predicted by fitting a linear regression of length vs. shell thickness to individuals from each population, and then calculating shell thickness. Differences in thickness between the two provinces and among populations were evaluated using a Mann-Whitney U test. Spinosity was assessed by examining mean number of spines per specimen, as well as by determining proportional shell width attributable to spines following the formula:

$$\text{Width added by spines} = \frac{(\text{width with spines} - \text{width without spines})}{\text{width with spines}}$$

Spinosity was compared among populations and between provinces to determine if differences were statistically significant using Kruskal-Wallis and Mann-Whitney tests, respectively. All statistical tests for linear morphometrics were completed in PAST (Hammer et al., 2001).

Repair Frequency

Many molluscan species record predatory attacks in their shells as repair scars (Figure 3.4), allowing for quantification of ecological pressures in both living and fossil specimens (Vermeij, 1987, 1993; Alexander & Dietl, 2003; Dietl, 2004). The opportunity to use repair scars as an estimate of predation pressure in molluscan studies allows the assessment of relative risk over large spatial scales without necessitating extensive experimental work. While there are many possible complications with using repair scars (e.g., variable growth rates and population size distributions can modify apparent frequencies [Schoener, 1979; Kowalewski, 2002; Alexander & Dietl, 2003]), they are widely accepted as an excellent tool for studying predation in both fossil and recent populations (see Kowalewski, 2002; Leighton, 2002; Alexander & Dietl, 2003 for discussion).

Figure 3.4. Examples of repair scars resulting from unsuccessful shell-crushing predation. Whelk specimen on left collected live in Wassaw Sound, Georgia: note repair scar (length=106.7 mm). Shell on right shows a repair scar from shell regeneration in the laboratory after an attack by a stone crab (length=107.2 mm).

Repair scars were used to compare predation levels between populations and regions (provinces). Repair scars that: 1.) cut across multiple growth intervals; or, 2.) represented jagged breaks extending over most of the length (>75%) of the apertural lip were classified as severe for this analysis, as minor breaks can result from self-induced wear due to feeding (Dietl, 2003a.) and other sources. Repair scars meeting these criteria represent Class 3 and higher damage in Figure 3.3. Variation in the frequency of shell repair (Figure 3.4) throughout *Busycon carica*'s range was conservatively calculated by dividing the number of individuals with at least one severe repair on their final whorl by the total number of individuals in the sample (Alexander & Dietl, 2003). Only individuals between 100-200 mm in length were used for this analysis.

Samples were standardized by size into 25 mm size classes (100-125 mm, >125-150 mm, >150-175 mm, and >175-200 mm). Binning of samples by size was necessary, as size may affect predator preference (e.g., size selective predation [Vermeij, 1987; Kitchell et al., 1981]), and thus the accumulation of repair scars (see Alexander & Dietl, 2003). Possessing a well-armored shell may change the rate of scar accumulation, therefore to minimize the effects of varying levels of shell armor, samples were further standardized by morphology to allow comparisons between individuals with the "northern" or weakly ornamented morphology (Figure 3.1) from both provinces. Samples were compared using a two-sample Chi-square test to examine differences between provinces for binned size classes. All statistical tests for repair frequency comparisons were completed in PAST (Hammer et al., 2001).

RESULTS

Predation Experiment

Differences in damage sustained for snails of different morphologies were significant ($p<.005$, $D=0.50$); northern morphologies ($n=24$) had a mean damage score of 2.88, as compared to a mean score of 1.71 ($n=23$) for southern morphologies (Figure 3.5). Survival frequency was significantly different between the two morphologies ($p=0.03$, Chi-square=6.72). Ten out of 24 northern knobbed whelks suffered fatal injuries during or in the two weeks following predation experiments, compared with only two out of 23 southern individuals (Figure 3.5). Individuals with thinner shells that lacked a tumid ridge were both more likely to suffer fatal injuries, and more likely to suffer severe damage as compared to the southern morphologies. Most of these differences in damage can be attributed to changes in shell thickness and the presence of the tumid ridge, with the southern morphology more strongly expressing anti-predatory traits. Spines were not observed to deter crab predators, and on several occasions seemed to give crabs better purchase on their preys' shells (as noted for other gastropods [Palmer, 1979]). Shell thickness was significantly different at the tumid ridge and shoulder between the two morphologies ($p<0.005$, $z=-4.81$; $p<0.005$, $z=-2.959$ for ridge and shoulder, respectively), and was thickest in southern, well-armored individuals (individuals possessing a tumid ridge, few large spines, and more massive shells). Average thicknesses at the shoulder and tumid ridge for weakly armored northern morphologies (lacking a tumid ridge, possessing several small spines) were 1.79 (standard error 0.11) and 2.01 (standard error 0.11) mm respectively, for southern morphologies thickness averaged 2.32 (standard error 0.13) and 3.32 (standard error 0.18) mm.

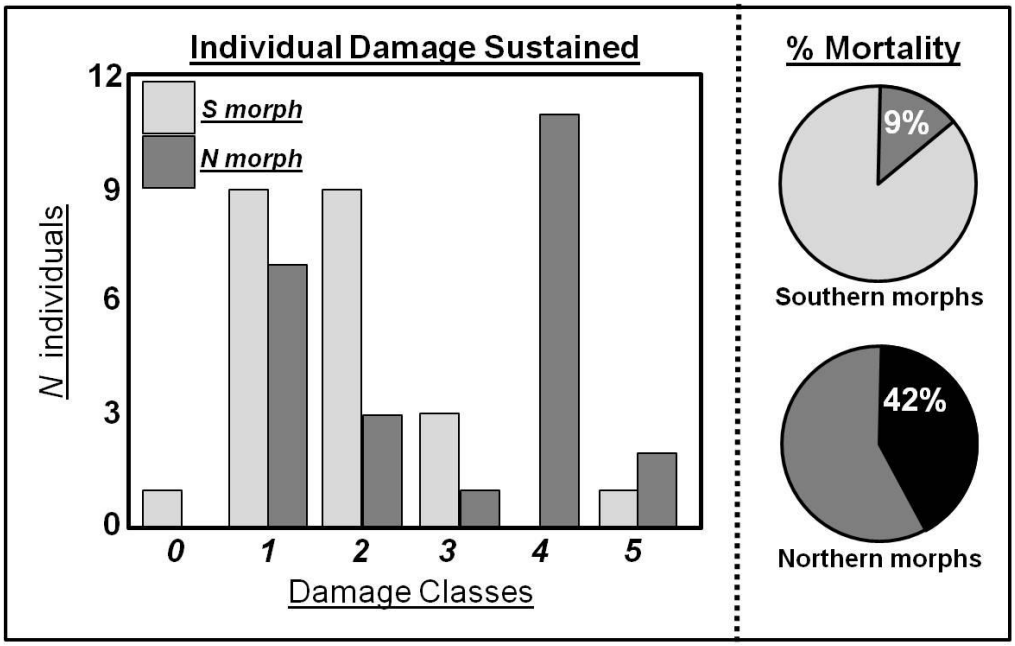

Figure 3.5. Predation experiment results. Left panel shows histogram of relative amounts of damage sustained in each damage category for southern (S) and northern (N) morphs; right panel shows proportional mortality out of total individuals for each morphology.

Handling behavior varied both between crabs, and within an individual's record of attacks. Different crabs showed preferences for particular orientations (e.g., canal up vs. spire up), and varied in where force was applied in attempts to break shells (e.g., crusher claw positioned across the aperture vs. across the spire). All crabs switched behavior several times over 4-hour predation intervals, with behavioral switches marked by changes in how the shell was oriented or how the crusher claw was oriented relative to the shell, and all crabs also switched behaviors throughout their ten predation attempts. Occasionally during predation series, I observed anecdotally that a "novel" behavior (a behavior initially observed in one trial) would be repeated in subsequent trials. For example, one crab initially did not attempt to crush across the canal of whelks, yet after the fourth experimental trial used this behavior in three successive attempts. Another crab incorporated canal crushing behavior in trials 6 and 8,

whereas another crab showed switches between predominantly attempting to crush across the body whorl vs. crushing across the spire.

Linear Morphometrics

Average size differs geographically, with larger individuals in the northern province (mean of 162 mm in the north [SE=2.17], compared to a mean of 144 mm in the south [SE=1.73], Appendix 3.1). Differences between provinces (Figure 3.6) were statistically significant ($z = -6.29$, $p<.0001$), and differences between populations were also significant in most cases ($H=189.3$, $p= 8.42E-33$). Mean lengths for individual localities varied from a high of 182 mm at Locality 6 (Rehobeth, Delaware) to a low of 118 mm at Locality 12 (Sapelo Island, Georgia) (SE=4.95)(Figure 3.6; Appendix 3.1). Averaged size for the five largest specimens showed similar trends. Thickness predicted at the tumid ridge for a 150 mm specimen varied substantially both between and within localities, though it was on average higher at localities south of Cape Hatteras (2.41 mm vs. 1.59 mm in the northern province, SE=0.20) (Figure 3.6; Appendix 3.1). Thickness at the shoulder showed a similar pattern, with a predicted thickness at a length of 150 mm of 2.66 mm in the northern province vs. a predicted thickness of 3.06 mm in the southern province (SE=0.18) . Differences between populations in the two provinces were statistically significant (Mann Whitney U for ridge thickness: $z=-2.49$, $p=0.01$; Mann Whitney U for shoulder thickness: $z=-2.72$, $p=0.007$). The tumid ridge only occurred in populations south of Cape Hatteras. Spinosity was highly variable, ranging between 1.75% of body width at Locality 1 (Wood's Hole, Massachusetts) to over 30% at Locality 10 (Wassaw Sound, GA). Spinosity was significantly higher in the southern province ($p<0.0001$, $U=2124$; means of 7.3% (N=201) for northern provinces [SE=0.32] and 21.8% (N=236) for southern provinces [SE=0.48]). Differences in the mean number of spines (Figure 3.6; Appendix 3.1) were also

highly significant between the two provinces ($p<0.0001$, $z=-9.60$), ranging between 7 and 11 with a mean of 10.9 (SE= 0.13, N= 193) in the north and a mean of 8.8 (SE=0.12, N=236) in the south.

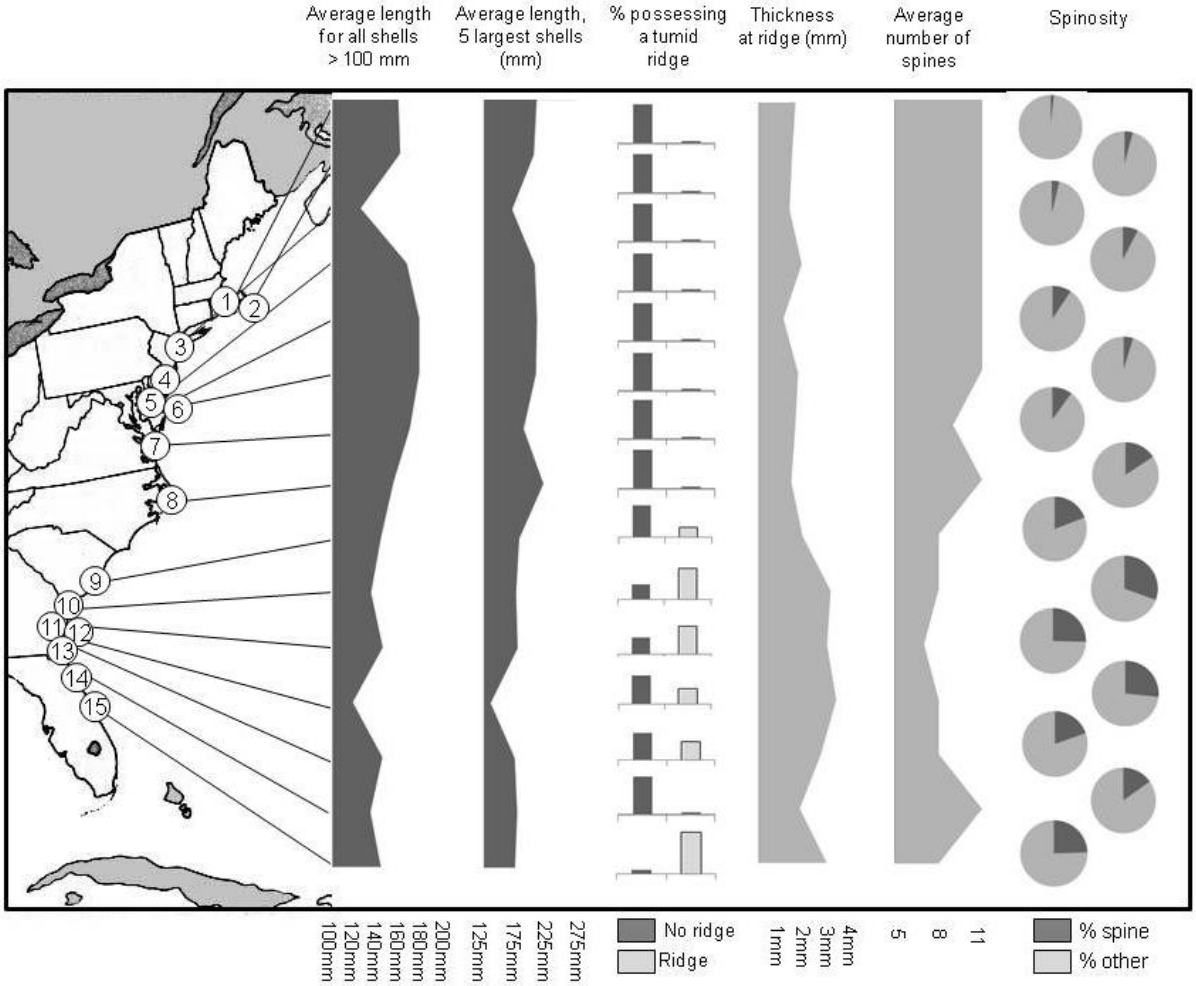

Figure 3.6. Distribution of morphological traits (spinosity, length, etc.) along cline.

Repair Frequency

Repair frequency data was variable both between provinces and between size classes. In all size classes except the largest, repair frequency was highest for weakly-armored morphologies in southern populations (Figure 3.7**).** None of the differences in repair scar

frequencies were statistically significant. Repair scar frequencies in this species are likely affected by geographic variation in growth rates throughout *B. carica's* range (see Discussion).

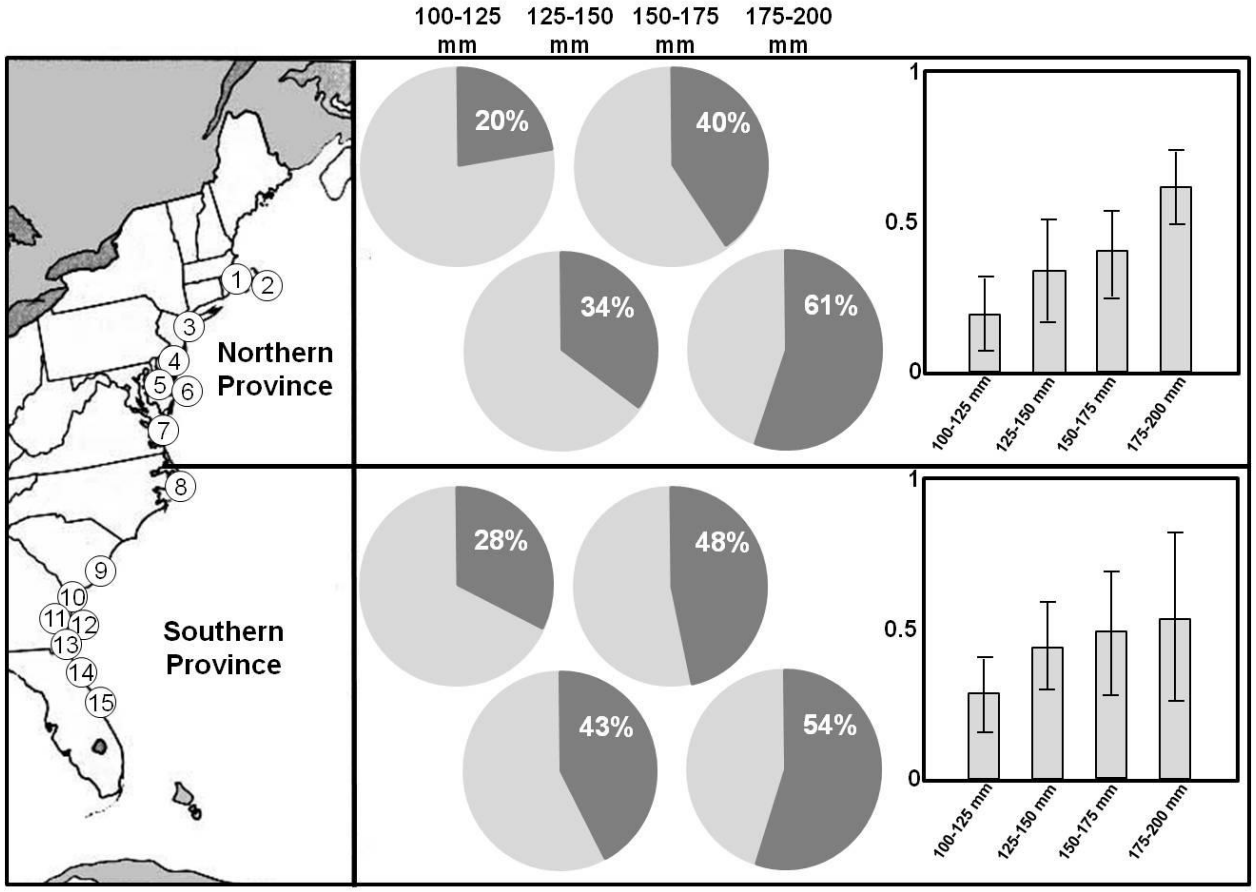

Figure 3.7. Repair scar frequency comparisons for different size classes in the northern and southern provinces. Repair frequency calculated as percentage of individuals in the population possessing at least one severe repair scar on their final whorl. Histograms to the right show repair frequencies with standard error bars.

DISCUSSION

Geographic clines in morphology

This study provides strong support for the theory that predation pressure intensifies at lower latitudes in marine communities. Southern populations of *Busycon carica* had greater

development of anti-predatory adaptations (i.e., the tumid ridge, increased shell thickness, and increased spinosity) than northern populations. Laboratory experiments strongly suggest that features presumed to be adaptive (increased shell thickness, the presence of a tumid ridge) provide fitness benefits in encounters with durophagous predators, though spines do not appear to confer anti-predatory benefits against stone crabs. Increases in proportions of well-armored individuals within populations occur in concert with an increase in durophagous predation, as evidenced by repair scar data; however, differences in repairs are non-significant. The incorporation of two distinct biogeographic provinces and the large spatial extent of this study (~ 10 degrees latitude) strengthens support for the hypothesis that predation pressure increases towards the tropics.

Shell thickness measured at the tumid ridge and shoulder peaks in the southernmost populations. Edwards (1988) reported a similar trend between five localities for *B. carica*, with increased shell mass peaking to the south. Increased shell thickness is a critical defense against durophagous predators, and even small differences may determine whether an individual survives or dies in an encounter with a durophagous predator. Despite the importance of a thick shell as a defense against shell-crushing predators, the correlation between length and thickness was typically low. Some variability in shell thickness, however, is expected: whelks are indeterminate growers, and add to their shells episodically. Single growth intervals are represented by additions of a few millimeters to several centimeters added at the lip. New growth is initially very thin (less than 1 mm), and thickens gradually over several months (Dietl, 2003b.). Thus shell thickness may vary dramatically depending on where in the growth cycle it is measured, which resulted in the observed low correlations between shell thickness and length

(R^2 values within populations ranged from less than 0.05-0.20 at the ridge, and from 0.15 to 0.61 at the shoulder).

A tumid ridge, which is only present in populations south of Cape Hatteras, is an effective, localized shell-thickening defense against crushing predators (specifically crabs), as was demonstrated in my predation experiments. The presence of a tumid ridge both increases survival odds and decreases the amount of damage sustained. However, this feature never entirely sweeps southern populations. The presence of weakly-armored individuals in southern populations may reflect the observed high spatial variation in predation intensity (repair frequencies ranged from 0.17 to 0.68 within the southern province). Low predation pressure in some populations would reduce selection for well-armored individuals, shifting morphologies towards a weakly-armored phenotype. The tumid ridge also appears to have a high associated cost. Preliminary data (Chapter 2) suggest that individuals possessing this trait grow more slowly than individuals lacking this and other adaptations, such as long spines: growth rates derived from isotope sclerochronology (e.g. Jones & Allmon, 1995; Dietl et al., 2002, Chapter 3) suggest that individuals possessing a tumid ridge take 5 to 7 years to reach a length of 100 mm, whereas weakly armored individuals only take 3.25-3.5 years to reach this length. Similar patterns have been observed in *Strombus gigas* (queen conch) juveniles, which grow more slowly and produce thicker shells when reared with predators, and in the muricid gastropod *Nucella lamellosa*, which shows greater spiral growth in thinner-shelled snails (Delgado et al., 2002; Palmer, 1981).

Spines shifted from being present as large protrusions accounting for roughly 25% of the total shell width in the south, to numerous small spines accounting for often less than 10% of the total shell width in populations north of Cape Hatteras. This pattern is similar to Edwards'

observations (1988); however, Edwards primarily attributed the cline in spinosity to the effects of increased wave exposure and turbulence. Edwards also speculated that spines might serve as a defense against predators, but did not test this hypothesis. In experiments with stone crabs, spines did not seem to provide an effective defense, and often enabled crabs to more firmly grip across the spire of the shell. Lethal breakage occurred either as deeply embayed breaks on the lip, or as punctures on the body whorl, further supporting the hypothesis that whelk spines are not effective adaptations against crabs. In one Florida population (Locality 14, Amelia Island), however, spines were expressed much less strongly than in neighboring populations (Figure 3.6). The tendency to have few but large spines in southern populations likely reflects adaptation against gape-limited predators, such as loggerhead turtles (see discussion below).

Shell length did not follow the expected trend: I predicted that mean length would be highest in the south in response to increased predation pressure, but it surprisingly decreased in the southern provinceThe unexpected trend of decreased whelk body size in the southern part of the range may result from differences in life history of *B. carica* along the cline, where whelks grow rapidly and die young in areas of intense predation and live longer and grow more slowly in areas of less intense predation. High predation rates on juveniles are known to select for rapid growth and/or early maturity (Wilbur & Saleuddin, 1974; Walsh & Reznick, 2009). Growth rate comparisons between the weakly armored morphologies from the northern and southern province support this hypothesis: southern individuals grow more rapidly, and reach maturity at a younger age (Chapter 3; Kraeuter et al., 1989; Power et al., 2009).

Agents of selection other than biotic factors are also likely important. A growing literature suggests that ectotherms commonly show latitudindal clines in body size and growth rates within species that correlate with temperature differences (Munch & Salinas, 2009), with

larger and slower growing individuals occurring at higher latitudes (e.g., Frank, 1975; Olabarria & Thurston, 2003; Lee & Boulding, 2010). This relationship between cooler temperatures, decreased growth rate, and larger body size may influence whelk adaptation, complicating interpretations of trends based solely on biotic factors. Further studies are needed to determine how biotic and abiotic environmental factors (agents of selection) might interact in shaping the evolution of *B. carica*.

Predation Experiments

The tendency of crabs to shift to and retain new behaviors throughout subsequent predation trials suggests a capacity to learn. Preliminarily, my data suggest that stone crabs have higher behavioral variability about a mean (either more or fewer shifts) when dealing with southern morphs. The one sinistral (left-handed) crab that was used, though it initially seemed to struggle to manipulate dextral whelks, lethally damaged 3 whelks over the course of ten trials. This result may be relevant for the ecology of *Busycon's* sister subgenus, *Sinistrofulgur*. As suggested by their name, *Sinistrofulgur* are left-handed but otherwise morphologically similar to *Busycon*. Opposite chirality prey have an advantage against predators, which prefer prey of the same handedness (Dietl & Hendricks, 2006). *Sinistrofulgur's* range overlaps partially with *Busycon carica*'s and populations show similar morphological variation, but different morphologies are spatially shifted. In regions where *Busycon carica* and *Sinistrofulgur* co-occur, *Busycon* possesses heavily armored shells in contrast to *Sinistrofulgurs'* weakly armored shells. *Sinistrofulgur* only develops well-armored shells in the Yucatan Peninsula (Wise et al., 2004). This distribution suggests that where the two species co-occur, *Sinistrofulgur* may experience a release from predation pressure as a result of right-handed durophagous predators preferring right-handed prey. *Busycon carica* is not present in the Yucatan; perhaps crabs in this region

have adapted by learning to handle sinistral prey. This presents an interesting avenue for future research.

Repair Scars

Repair scar frequencies for the size- and morphologically- standardized data peaked in the southern province, as predicted, for all but the largest size class (175-200 mm). Standardization by morphology is a new technique which may help capture ecologically relevant signals. Laboratory experiments suggest that the two morphologies differ in their potential to accumulate shell repairs, which makes consideration of morphology particularly important when interpreting repair frequencies. Within the southern province, weakly armored individuals (low spinosity, lacking a tumid ridge) had fewer repair scars in the two smaller size classes (<150 mm) when compared to well armored individuals. Predation experiments predict these results, as weakly armored shells experienced high mortality rates, which would prevent the accumulation of repair scars. Well armored individuals are more likely to survive predatory attacks at small body sizes, resulting in the noted increase in repair scar frequencies. The function of the tumid ridge and increased shell thickness, then, is not in deterring predators from attacking, but instead defending a shell that will inevitably experience an attack.

Growth rates, derived from stable isotopes as well as from prior work on *Busycon* (Chapter 3; Kraeuter et al., 1989; Power et al., 2009), indicate that growth rate varies substantially with latitude, with higher growth rates to the south. Currently, the paucity of studies (particularly to the north) and different treatment of data across growth rate studies (e.g., exclusion of individuals with negative or non-growth [Bruce et al., 2008], inconclusive division of genders [Kraeuter et al., 1989], combination of laboratory reared vs. mark-recapture vs. estimates derived from opercula) make it difficult to assess the exact rates of growth within

provinces; however, the general latitudinal trend is likely to hold. With further studies encompassing more individuals and more localities, standardizations of repair frequency by growth rates (considering an annual rate of repair scar accumulation, for example) may become possible. Prior studies suggest that accounting for more rapid growth to the south will further increase repair frequency, and thus estimates of predation pressure.

Other predators

Individuals that possess a tumid ridge do not always possess large spines, and vice versa. One possible explanation is that the two features may be adaptations against different predators. The loggerhead turtle (*Caretta caretta*) is known (from gut contents) to consume *Busycon carica* in the wild (Wallace et al., 2009; McClellan et al., 2010), and whelks may make up a significant portion of their diet in parts of their range (up to 50% combined blue crabs and whelks, Wallace et al., 2009). While juvenile *C. caretta* occupy pelagic habitats, as individuals age they shift towards more coastal feeding grounds where they consume large amounts of benthic invertebrates (Hopkins-Murphy et al., 2003; Witherington, 2003). While there are no reported direct observations of turtles feeding on whelks, it seems unlikely that the possession of a tumid ridge would function effectively as an anti-predatory adaptation against loggerhead turtles. Loggerhead turtles, which possess powerful jaws well-suited for feeding on hard-shelled mollusks and crabs (Kamezaki, 2003), could likely easily crush whelks either with or without tumid ridges. It seems more likely that whelk spines, which increase the effective width of whelks, might function as protection against these or other gape-limited predators, as noted in prior studies (Palmer, 1979). Nesting grounds in the United States occur south of Cape Hatteras, with an estimated 90% of nest sites in Florida (Bowen, 2003), so predation pressure from loggerheads should be highest in the southern province. De-coupling of spines from the tumid

ridge, as observed with individuals from Locality 14 (Amelia Island, Florida), may be an adaptive response to high turtle predation and low stone crab predation.

Plasticity

I did not test whether clinal morphological differences in *B. carica* result from adaptation or phenotypic plasticity due to the long time to maturity for this species: ages at 100 mm ranged from 3.25 to 7 years (Chapter 3). Given these growth rates, it is highly unlikely that developing a well-armored morphology could serve effectively as a plastic response to predation. Whelks in this study rarely added to their shells even after severely damaging attacks, and using growth estimates derived from the Georgia and New York whelks, defenses would take in excess of a year to develop to an effective level (i.e., development of a tumid ridge that wraps around the body whorl). The lengthy time required to develop this adaptation strongly suggests that it could not function as an inducible defense.

CONCLUSIONS

The results of predation experiments, as well as the distribution of adaptive traits in the wild, support my initial hypothesis that predation pressure drives the maintenance of increased anti-predatory adaptations in the southern part of *Busycon*'s range. This study provides both an experimental test of whether or not different traits (increased shell thickness, large spines, and presence of a tumid ridge) serve as anti-predatory adaptations, as well as a comprehensive assessment of both predation pressure and the distribution of different morphologies throughout the 10 degrees of latitude that comprise *B. carica*'s range. Laboratory experiments comparing the efficacy of the two different morphologies at deterring predators suggest that systematic

differences in shell shape along the eastern coast of the United States are the result of local adaptation in the southern province to a host of powerful durophagous predators.

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APPENDIX

Appendix 3.1.

Locality	Latitude	*N*	Average Length, >100 mm	Average Length, 5 Largest Specimens	Spines as % shell width	Number of Spines	Predicted thickness at tumid ridge, 150 mm	Predicted thickness at shoulder, 150 mm	Museum Specimen Information
1.Wood's Hole, MA	41.53	21	162.53	211.15	1.75	11	1.65	1.78	DMNH 120672, NMNH 590047, NMNH 1021638
2. Nantucket, MA	41.28	17	164.16	206.21	4.14	11	1.49	1.76	NMNH 592026, NMNH 590648, NMNH 36299, PRI Acc. 2810c, PRI Acc. 44A Bentley Collection
3. West Neck, NY	40.80	61	126.98	171.42	3.62	11	1.38	1.99	PRI Acc. 100
4. Stone Harbor, NJ	39.00	30	170.40	208.11	7.56	11	1.92	2.87	G. Dietl Coll.
5. Cape Henlopen, DE	38.77	51	182.05	211.73	9.34	11	1.11	1.55	NMNH 857001, NMNH 847010, NMNH 836998, NMNH 845555, NMNH 806863, NMNH 836991
6. Rehobeth, DE	38.72	30	182.08	210.03	4.71	11	1.76	2.34	DMNH 202343, DMNH 213492
7. Cape Charles, VA	37.25	14	173.66	190.24	9.92	9	1.63	2.40	NMNH 874987
8. Beaufort, NC	34.70	71	157.84	222.04	15.57	11	1.46	2.60	PRI Acc. 1472
9. Charleston, SC	32.80	28	146.14	182.88	19.19	8	1.96	2.73	NMNH 806861, NMNH 857012, NMNH 806880
10. Wassaw Sound, GA	31.93	47	136.78	177.77	30.50	8	3.20	3.75	M. Kosloski Coll.
11. St. Catherine's Island, GA	31.63	44	147.84	180.34	25.44	7	3.04	3.35	G. Dietl Coll.
12. Sapelo Island, GA	31.39	40	119.54	136.57	26.65	8	3.45	3.86	M. Kosloski Coll.
13. St. Simon's Island, GA	31.21	35	147.45	175.57	19.60	8	2.77	3.12	NMNH 857008, NMNH 857005
14. Amelia Island, FL	30.61	17	136.19	179.65	15.14	11	1.83	2.42	NMNH 806881, NMNH 55684, NMNH 83293
15. Cape Canaveral, FL	28.39	12	145.99	175.99	24.47	8	3.03	2.68	NMNH 807502, NMNH 515221, NMNH 845556

Appendix 3.1. Localities, population statistics, and sample information.
DMNH=Delaware Museum of Natural History, NMNH=Smithsonian Museum of Natural History, PRI=Paleontological Research Institution

REFERENCES

Abbott, R.T. 1954. American Seashells. Van Nostrand Reinhold Co., New York. 652 pp.

Abbott, R. T. 1974. American Seashells. 2nd Edition. Van Nostrand Reinhold Co., New York. 663 p.

Alexander, R. R., & G. P. Dietl. 2003. The Fossil Record of shell-breaking predation on marine bivalves and gastropods. In Predator-Prey Interactions in the Fossil Record, Eds. Kelley, P. H., Kowalewski, M. & Hanson, T. A. Kluwer Academic/Plenum Publishers, New York.

Appleton, R.D., & A.R. Palmer. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proc. Natl. Acad. Sci. 85, 4387-4391.

Behrens Yamada, S. and E.G. Boulding. 1998. Claw morphology, prey size selection, and foraging efficiency in generalists and specialist shell-breaking crabs. Journal of Experimental Marine Biology and Ecology 220:191-211

Berlocher, S. H. 2000. Allozyme variation in *Busycon* Whelks (Gastropoda: Melongenidae). Biochemical Genetics38: 285-295.

Bert, T. M. 1986. Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological processes and climatic events in the formation and distribution of species. Marine Biology 93:157-170.

Bert, T.M., & R. G. Harrison. 1988. Hybridization in western atlantic stone crabs (genus Menippe): evolutionary history and ecolog- ical context influence species interactions. Evolution 42: 528–544

Bertness, M.D., Garrity, S.D., & S.C. Leavings. 1981. Predation pressure and gastropod foraging: a tropical-temperate comparison. Evolution 35: 995-1007.

Blundon, J.A. 1988. Morphology and muscle stress of chelae of temperate and tropical stone crabs *Menippe mercenaria*. J. Zool. London 215:663-673.

Bolser, R.C., & M.E. Hay. 1996. Are tropical plants better defended? Palatability and defenses of temperate versus tropical seaweeds. Ecology 77: 2269-2286

Bowen, B.W. 2003. What is a loggerhead turtle? The genetic perspective. . In: A. B. Bolten & B. E. Witherington (eds.), Loggerhead Sea Turtles. Smithsonian Books, Washington D.C. 319 pp.

Brookes J.I., & R. Rochette. 2007. Predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*: developmental by-product or active physiological response? Journal of Evolutionary Biology 20: 1015-1027.

Carriker, M.R., 1951. Observations on the penetration of tightly closing bivalves by *Busycon* and other predators. Ecology 32: 73–83.

Cody, M.L. 1966. A general theory of clutch size in birds. Evolution 20:174-190.

Coley, P.D. & J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27: 305-335.

Colton, H.S. 1908. How *Fulgur* and *Sycotypus* eat oysters, mussels and clams. Proc. Acad. Nat. Sci. Philadelphia 60: 3–10.

Dalziel, B. & E.G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology 317: 25-35.

De Jong, G. & Z. Bochnadovits. 2003. Latitudinal clines in Drosophila melanogaster: body size, allozyme frequencies, inversion frequencies, and the insulin-signalling pathway. Journal of Genetics 82:207-233.

Delgado, G. A., Glazer, R. A., & N.J. Stewart. 2002. Predator-Induced Behavioral and Morphological Plasticity in the Tropical Marine Gastropod *Strombus gigas*. Biological Bulletins 203:112-120.

Dietl, G. P. 2003a. Coevolution of a marine gastropod predator and its dangerous bivalve prey. Biol. J. Linn. Soc. 80:409–436.

Dietl, G. P. 2003b. Interaction strength between a predator and dangerous prey : *Sinistrofulgur* predation on *Mercenaria*. Journal of Experimental Marine Biology and Ecology289:287 - 301. doi:10.1016/S0022-0981(03)00047-9

Dietl, G. P. 2004. Origins and circumstances of adaptive divergence in whelk feeding behavior. Palaeogeography, Palaeoclimatology, Palaeoecology 208:279-291.

Dietl, G. P. 2009. Paleobiology and the conservation of the evolving web of life. In G. P. Dietl and K. W. Flessa (eds.) Conservation Paleobiology: Using the Past to Manage for the Future. Paleontological Society Special Publication Vol. 15: 221-244.

Dietl, G. P. & J.R. Hendricks. 2006. Crab scars reveal survival advantage of left-handed snails. Biology Letters 2:439-442.

Dietl, G. P., Kelley, P. H., Barrick, R. and W. Showers. 2002. Escalation and extinction selectivity: morphology versus isotopic reconstruction of bivalve metabolism. Evolution 56: 284–291. doi: 10.1111/j.0014-3820.2002.tb01338.x

Edwards, A. L. 1988. Latitudinal clines in shell morphologies of *Busycon carica* (Gmelin 1791). Journal of Shellfish Research 7(3):461-466.

Edwards, A. L. & M.G. Harasewych. 1988. Biology of the Recent Species of the Subfamily Busyconinae. Journal of Shellfish Research 7:467-472.

Edwards, A. L. & C.M. Humphrey. 1981. An electrophoretic and morphological survey of *Busycon* occurring in Wassau Sound, Georgia. Nautilus 95:144

Ehrhart, L.M., Bagley, D.A., & W.E. Redfoot. 2003. Loggerhead turtles in the Atlantic Ocean: geographic distribution, abundance, and population status. In: A. B. Bolten & B. E. Witherington (eds.), Loggerhead Sea Turtles. Smithsonian Books, Washington D.C. 319 pp.

Engle, V.D. & J.K. Summers. 1999. Latitudinal gradients in benthic community composition in Western Atlantic estuaries. Journal of Biogeography 26:1007-1023.

Frank, P.W. 1975. Latitudinal variation in the life history features of the black turban snail *Tegula funebralis* (Prosobranchia: Trochidae). Marine Biology 31: 181-192.

Freestone, A.L., Osman, R.W., Ruiz, G.M.,& M. E. Torchin. 2011. Stronger predation in the tropics shapes species richness patterns in marine communities. Ecology 92:983–993. [doi:10.1890/09-2379.1]

Griffiths A.M., & L.A. Gosselin. 2008. Ontogenetic shift in susceptibility to predators in juvenile northern abalone, *Haliotis kamtschatkana*. Journal of Experimental Marine Biology and Ecology 360: 85-93

Halpern, B. S., Selkoe, K.A., Micheli, F., & C.V. Kappel. 2007. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conservation Biology 21:1301-15 doi:10.1111/j.1523-1739.2007.00752.x

Hammer, Ø., Harper, D.A.T., & P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 9pp.

Hellberg, M.E., Balch, D.P., & K. Roy. 2001. Climate-driven range expansion and morphological evolution in a marine gastropod. Science 292: 1701-1710.

Hollister, S. C. 1958. A Review of the Genus *Busycon* and its allies- Part I. Paleontographica Americana 4:49-126.

Hopkins-Murphy, S.R., Owens, D.W., & T.M. Murphy. 2003. Ecology of immature loogerheads on foraging grounds and adults in interesting habitat in the eastern United States. In: A. B. Bolten & B. E. Witherington (eds.), Loggerhead Sea Turtles. Smithsonian Books, Washington D.C. 319 pp.

Hoso, M., Asami, T. & M. Hori. 2007. Right-handed snakes: convergent evolution of asymmetry for functional specialization. Biology Letters 3:169-172.

Hughes, A.R. & J.H. Grabowski. 2006. Habitat context influences predator interference interactions and the strength of resource partitioning. Oecologia149:256-264.

Inoda, T., Hirata, Y. & S. Kamimura. 2003. Asymmetric mandibles of water-scavenger larvae improve feeding effectiveness on right-handed snails. American Naturalist 162:811-814.

Jackson, J. B., Kirby, M. X., Berger, W. H., Bjorndal, K. A, Botsford, L. W., Bourque, B. J., & R.H. Bradbury. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-37. doi:10.1126/science.1059199

Jones, D. S. & W.D. Allmon. 1995. Records of upwelling, seasonality and growth in stable-isotope profiles of Pliocene mollusk shells from Florida. Lethaia 28: 61–74. doi: 10.1111/j.1502-3931.1995.tb01593.x

Kamezaki, N. 2003. What is a Loggerhead turtle? The morphological perspective. In: A. B. Bolten & B. E. Witherington (eds.), Loggerhead Sea Turtles. Smithsonian Books, Washington D.C. 319 pp.

Kari, J.S. & R.B. Huey. 2000. Size and seasonal temperature in free-ranging *Drosophila subobscura*. Journal of Thermal Biology 25: 267-272.

Kent, B.W., 1983. Patterns of coexistence in Busyconine whelks. J. Exp. Mar. Biol. Ecol. 66: 257–283.

Kitchell, J.A., Boggs, C.H., Kitchell, J.F., & J.A. Rice. 1981. Prey selection by naticid gastropods: experimental tests and application to the fossil record. Paleobiology 7: 533-552.

Kowalewski, M. 2002. The fossil record of predation: an overview of analytical methods. In: M.

Kowalewski and P. H. Kelley (eds.), The Fossil Record of Predation. The Paleontological Society Papers, 8: Pp. 3-42.

Kraeuter, J.N., Castagna, M., Bisker, R. 1989. Growth rate estimates for *Busycon carica* (Gmelin, 1791) in Virginia. Journal of Shellfish Research 8(1):219-225.

Krantz, D.E. 1990. Mollusk-Isotope Records of Plio-Pleistocene Marine Paleoclimate, U.S. Middle Atlantic Coastal Plain. Palaios 5(4):317-335.

Laurila, A., Lindgren, B., & A.T. Laugen. 2008. Antipredator defenses along a latitudinal gradient in *Rana temporaria*. Ecology 89:1399-1413.

Lee, H.J., & E.G. Boulding. 2010. Latitudinal clines in body size, but not in thermal tolerance or heat-shock cognate 70 (*HSC70*), in the highly-dispersing intertidal gastropod *Littorina keenae* (Gastropoda: Littorinidae). Biological Journal of the Linnean Society 100: 494-505.

Leighton, L.R. 2002. Inferring predation intensity in the marine fossil record. Paleobiology 28 (3) pp. 328-342.

Magalhaes, H. 1948. An ecological study of snails of the genus *Busycon* at Beaufort, North Carolina. Ecological Monographs 18, 377-409.

McClellan, C.M., Braun-McNeill, J., Avens, L., Wallace, B.P., & A.J. Read. 2010. Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. Journal of Experimental Marine Biology and Ecology 387: 44-51.

Mitton, J.B. 1977. Shell color and pattern variation in *Mytilus edulis* and its adaptive significance. Chesapeake Science 18:387-390.

Munch, S.B., & S. Salinas. 2009. Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. PNAS 106 (33), 13860-13864.

Olabarria, C., & M.H. Thurston. 2003. Latitudinal and bathymetric trends in body size of the deep-sea gastropod *Troschelia berniciensis* (King). Marine Biology 143: 723-730.

Paine, R.T., 1962. Ecological diversification in sympatric gastropods of the genus *Busycon*. Evolution 16: 515–523.

Paine, R.T., 1976. Size-Limited Predation: An Observational and Experimental Approach with the *Mytilus-Pisaster* Interaction. Ecology 7: 858-873.

Palmer, A.R. 1979. Fish predation and evolution of gastropod shell sculpture: experimental and geographic evidence. Ecology 33:697-713.

Palmer, A.R. 1981. Do carbonate skeletons limit the rate of body growth? Nature 292: 150-152.

Palmer, A.R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). Hydrobiologia 193:155-182.

Petuch, E. J. 1994. Atlas of Florida Fossil Shells (Pliocene and Pleistocene Marine Gastropods) Chicago Spectrum Press, 394 pages

Pennings, S.C., E.L. Siska, & M.D. Bertness. 2001. Latitudinal differences in plant palatability in Atlantic Coast salt marshes. Ecology 82: 1344-1359.

Pennings, S.C. & B.R. Silliman. 2005. Linking biogeography and community ecology: latitudinal variation in plant-herbivore interaction strength. Ecology 86 : 2310-2319.

Pennings, S.C., Ho, C., Salgado, C.S., Więski, K., Davé, N., Kunza, A.E., & E.L.Wason. 2009. Latitudinal variation in herbivore pressure in Atlantic Coast salt marshes. Ecology 90:183–195. [doi:10.1890/08-0222.1]

Pianka, E. R. 1966. Latitudinal gradients in species diversity: a review of concepts. American Naturalist 100:33-46.

Power, A.J., Sellers, C.J., & R.L. Walker. 2009. Growth and sexual maturity of the knobbed whelk, *Busycon carica* (Gmelin 1791), from a commercially harvested population in coastal Georgia. Occasional papers of the University of Georgia Marine Extension Service, Vol. 4, 24 pp.

Randall, J. 1964. Contributions to the biology of the queen conch, *Strombus gigas*. Bulletin of Marine Science 14: 246-295.

Roy, K., Collins, A.G., Becker, B.J., Begovic, E., & J.M. Engle. 2003. Anthropogenic impacts and historical decline in body size of rocky intertidal gastropods in southern California. Ecology Letters 6: 205-211.

Scavia, D., Field, J.C., Boesch, D.F., Buddemeier, R.W., Burkett, V., Cayan, D.R., Fogarty, M., Harwell, M.A., Howarth, R.W., & C. Mason. 2002. Climate change impacts on U.S. Coastal and Marine Ecosystems. Estuaries and Coasts 25 : 149-164.

Schemske, D. W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M., & K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions? Annual Review of Ecology, Evolution and Systematics 40: 245-269.

Schenck, S.C. & P.C. Wainwright. 2001. Dimorphism and the functional basis of claw strength in six brachyuran crabs. J. Zool. London 255:105-119.

Schmidt, P.S., Serrao, E.A., Pearson, G.A., Riginos, C., Rawson, P.D., Hilbish, T.J., Brawley, S.H., Trussell, G.C., Carrington, E., Wethey, D.S., Grahame, J.W., Bonhomme, F., & D.M. Rand. 2008. Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci. Ecology 89 (11 suppl): S91-107.

Schoener, T.W. 1979. Inferring the properties of predation and other injury-producing agents from injury frequencies. Ecology 60:1110-1115.

Seeley, R.H. 1986. Intense natural selection caused a rapid morphological transition in a living marine snail. Proceedings of the National Academy of Sciences 83: 6897-6901.

Simonson JL. 1985. Reversal of handedness, growth, and claw stridulatory patterns in the stone crab *Menippe mercenaria* (Say) (Crustacea: Xanthidae). Journal of Crusteacean Biology 5:281-293.

Stachowicz, J.J. & M.E. Hay. 2000. Geographic variation in camouflaging behavior by the decorator crab *Libinia dubia*. American Naturalist 156:59-71.

Thompson, J.N. 2005. The Geographic Mosaic of Coevolution, Chicago, University of Chicago Press. 400 pp.

Toju, H., & T. Sota. 2006. Imbalance of predator and prey armament: geographic clines in phenotypic interface and natural selection. The American Naturalist 167 1: 105-117.

Trussell, G.C. & R.J. Etter. 2001. Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. Genetica 112-113:321-337.

Trussell, G.C. & L.D. Smith. 2000. Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. PNAS 97(5) 2123-2127.

Trussell, G.C. & M.O. Nicklin. 2002. Cue sensitivity, Inducible defense, and trade-offs in a marine snail. Ecology 83 (6)1635-1647.

Vermeij, G.J., Dietl, G.P., & D.G. Reid. 2008. The Trans-Atlantic history of diversity and body size in ecological guilds. Ecology 89 (11), supplement pp. s39-s52.

Vermeij, G. J. 1977. Patterns in crab claw size: the geography of crushing. Systematic Zoology, 26:138-151.

Vermeij, 1978. Biogeography and adaptation: patterns of marine life. Harvard University Press, Cambridge, Massachusetts, USA. 332 pp.

Vermeij, G. J. 1987. Evolution and escalation, an ecological history of Life. Princeton University Press, Princeton, New Jersey. 544 pp.

Vermeij, G. J. 1993. A Natural History of Shells. Princeton University Press, Princeton, New Jersey. 216 pp.

Wallace, B.P., Avens, L., Braun-McNeill, J., & C.M. McClellan. 2009. The diet composition of immature loggerheads: Insights on trophic niche, growth rates, and fisheries interactions. Journal of Experimental Marine Biology and Ecology 373: 50-57.

Walsh, M. R., & D.N. Reznick. 2009. Phenotypic diversification across an environmental gradient: a role for predators and resource availability on the evolution of life histories. Evolution; international journal of organic evolution. 63:3201-13. doi:10.1111/j.1558-5646.2009.00785.x

Warren, S., 1916. The feeding habits of *Busycon*. Nautilus 30: 66–68.

Wilber, K.M., and A.S.M. Saleuddin. 1983. Shell formation. Pages 236-287 in K.M. Wilber, ed. *The Mollusca*: Vol 4, Physiology, Part 1. New York Academic Press, New York. 523 p.

Wise, J., Harasewych, M.G., & R.T. Dillon Jr. 2004. Population divergence in the sinistral whelks of North America, with special reference to the east Florida ecotone. Marine Biology 145: 1167-1179.

Witherington, B.E. 2003. Biological Conservation of Loggerheads: Challenges and Opportunities. In: A. B. Bolten & B. E. Witherington (eds.), Loggerhead Sea Turtles. Smithsonian Books, Washington D.C. 319 pp.

Yamada, S.B. 1989. Are direct developers more locally adapted than planktonic developers? Marine Biology 103 3: 403-411.

Zuschin, M., Stachowitsch, M., & R. J. Stanton Jr. 2003. Patterns and processes of shell fragmentation in modern and ancient marine environments. Earth Science Reviews 63: 33-82.

CHAPTER 4

ANTI-PREDATOR MORPHOLOGY IN DEEP TIME: ECOMETRICS AND ADAPTATION IN *BUSYCON* AND *SINISTROFULGUR*

ABSTRACT

I applied ecometric techniques, in which the distribution of functional traits across communities and time is examined in a taxon-free context, to busyconine whelks from the Pliocene to the Recent. Many morphological traits (i.e., sinistrality, possession of a tumid ridge, possession of spines, increased shell thickness) correlate with enhanced defensive ability, and may be used as indicators of high-predation environments. During the Plio-Pleistocene, the western Atlantic experienced substantial faunal turnover associated with the closing of the Central American Seaway and resultant environmental changes. I tested the hypothesis that functional traits should be most abundant in the early to middle Pliocene, when conditions were likely more risky (i.e., higher predation). After the boundary, adaptations are expected to decrease in abundance in response to lower predation following the extinction event. Repair scar analyses provided an additional index of the strength of selection for the maintenance of anti-predatory adaptations across this time period. "Expensive" defensive traits (e.g., large spines, the tumid ridge, and thick shells) were most abundant prior to the Plio-Pleistocene boundary, with highest expression in Virginia for dextral whelks and in Florida for sinistral whelks. Overall expression of traits was strongest in dextral whelks, and both chiralities show decreased adaptations in the early Pleistocene. In the Recent, dextral whelks have a high incidence of adaptive traits, particularly in the southern part of their range, which may be indicative of faunal recovery following the extinction.

INTRODUCTION

The environmental and ecological settings associated with maintaining adaptive features are of much interest in evolutionary studies (e.g., Vermeij, 1987; 1993; Alexander & Dietl, 2003). Gastropods are a tractable organism for studying the distribution of morphological adaptations, as their shells provide an excellent fossil record of the history of shape change, predation, and with the application of sclerochronological techniques, climate, temperature, and oceanographic data. Many morphological adaptations in gastropods are costly, and incur trade-offs between alternate adaptations, reproduction, or growth (e.g., Geller, 1990; Palmer, 1990; DeWitt et al., 2000; Trussell & Etter, 2001; Delgado et al., 2002; Trussell & Nicklin, 2002; Brookes & Rochette, 2007). Costly features, which are expected to be both spatially and temporally limited in their expression, are of particular interest, as their appearance may be useful as an indicator of associated ecological parameters.

Ecometric techniques (Eronen et al. 2010, Polly et al. 2011) use a phylogenetic-free context to examine the history of adaptation by studying the distribution of functional traits across communities, and the environmental and ecological contexts in which they arise and are prevalent. Recently, these techniques have received attention for their utility in examining faunal responses to climate change, and their ability to integrate studies of ecomorphology (how traits relate to climate and the environment) with environmental change and the fossil record (i.e., Lawing et al., 2012). Some common applications include the use of mammalian dental characteristics to estimate rainfall and temperature (e.g., Liu et al., 2012) and the use of the wholeness vs. jaggedness of leaf margins to estimate paleo-temperature (e.g., Bailey & Sinnott, 1915, 1916; Wilf, 1997; Royer & Wilf, 2006).

I quantified the Pliocene to Recent history of several morphological adaptations known to confer anti-predatory benefits within busyconine whelks. An ecometric approach was used to integrate functional morphology of traits and their association with particular ecological or environmental conditions (in this case, traits associated with high predation risk) in deep time. Trait distribution was examined in conjunction with repair scar analyses, as repair scars could indicate the strength of selection for the maintenance of morphological adaptations (Vermeij, 1982). Adaptive traits are predicted to be most abundant in coincidence with highly escalated faunas, and thus expression of adaptations should be diminished in lower risk environments. Ecometrics were used to characterize the response of busyconine whelks to the extinction event and subsequent recovery.

STUDY SYSTEM

Busyconine whelks (Family Melongenidae, genus *Busycon* and subgenus *Sinistrofulgur*; Miocene-Recent) are an abundant and ecologically important component of shallow marine faunas along the U.S. Atlantic Coast, with several species ranging through to the modern (e.g., Magalhaes, 1948; Hollister, 1958; Harasewych, 1982; Edwards & Harasewych, 1988). *Busycon* displays a multitude of likely adaptive anti-predatory morphologies throughout its evolutionary history. Body size is variable within the modern species, *Busycon carica* (Abbott, 1974; Chapter 3), and increased body size may provide a refuge from predators (Paine, 1976; Vermeij, 1993). Increased shell thickness is also often correlated with increases in defensive performance, and may be one of the most effective defenses against crushing predatiors (Vermeij, 1993; Zuschin et al., 2003). Shell thickness varies considerably throughout the modern species' range

(Chapter 3). The **tumid ridge** (Hollister, 1958), a prominent swelling that runs along the outside of the body whorl and that is unique to this clade in the western Atlantic, appears repeatedly in geographically and temporally disjunct populations from the Pliocene to the Recent (Hollister, 1958; Kosloski & Dietl, 2011; Chapter 3). The genus also displays variable development of **spines** in space and time (e.g., Edwards, 1988), and shell thickness and length also vary across time and within modern populations.

Both spines and the tumid ridge have been hypothesized in the modern to provide individuals possessing them with anti-predatory fitness advantages (e.g., Edwards, 1988). Spines may be beneficial against gape-limited predators (e.g. turtles, rays, fish [Palmer, 1979]), as they increase effective shell width by up to 30% (Chapter 3). Laboratory experiments have demonstrated that the modern species, *Busycon carica*, performs significantly better in terms of both overall survival and amount of shell damage sustained against the stone crab, *Menippe mercenaria*, when it possesses a tumid ridge and thickened shell (Chapter 3). Additionally, the tumid ridge is most strongly developed and occurs in the highest frequencies in wild populations that co-occur with powerful durophagous predators, (particularly *Menippe mercenaria),* reinforcing laboratory studies that suggest that this feature has an anti-predatory function (Chapter 3).

The development and maintenance of the above adaptations in *B. carica* incur high costs. In modern populations, a well-ornamented morph grows substantially more slowly than a weakly-ornamented morph, even between individuals collected from the same population (Chapter 2). Costly features are predicted to be most abundant in escalated, risky environments (environments where competition and predation rates are high [Vermeij, 2001, 2007]), as these environments make the adaptation more valuable to possess. The loss or diminished expression

of a costly adaptive feature, on the other hand, implies a loss of defensive performance (Vermeij, 2007). The expression of costly adaptations should therefore decrease in times of lowered risk.

Sinistral coiling (sinistrality) is very rare among marine gastropods (Vermeij, 2002), and also appears in whelks in the Pliocene within the subgenus *Sinistrofulgur* (e.g., Dietl & Hendricks, 2006). Sinistrality persists in several species today (e.g., Hollister, 1958; Paine, 1962; Wise et al., 2004), and has been shown to confer an anti-predatory advantage when deterring predators of an opposite chirality in several modern gastropod species (e.g., Inoda et al., 2003; Hoso et al., 2007). Sinistral whelks in the Pliocene of the Atlantic Coastal Plain accumulated fewer repair scars than dextral whelks, suggesting an anti-predator advantage in the Pliocene as well (Dietl & Hendricks, 2006). Reversed chirality snails may show diminished expression of adaptations relative to dextral whelks that overlap in range, as sinistrality may diminish selection for adaptations by reducing predation pressure.

Busyconine Phylogeny and Systematics

While the genus *Busycon* has been well studied since the 1800s, there is currently no encompassing phylogenetic framework for the lineage (e.g., Harasewych, 1982; Edwards & Humphrey, 1981; Edwards & Harasewych, 1988). Many obvious shell morphological features, such as spinosity and presence or absence of the tumid ridge, are variable both within and between populations and grade from absent to present within species over their geographic range (Edwards, 1988). *Busycon*'s high level of morphological variation and its spatial partitioning have led several authors to abundantly split populations taxonomically at both the species and subspecies level, in the fossil record as well as the modern (e.g., Hollister, 1958; Petuch, 1991; 1994); however, genetic and morphological studies of Recent species (*Busycon carica* and *Sinistrofulgur sinistrum*) show extensive intergradation between morphologies, and suggest that

splitting below the species level cannot be justified (Abbott, 1974; Edwards and Humphrey, 1981; Harasewych, 1982; Edwards & Harasewych, 1988; Berlocher, 2000; Wise et al., 2004). Morphological variation has also long been apparent in fossil species (e.g., Leidy, 1889). The lack of easy-to-code morphological characters is a common issue in gastropod systematics (e.g., Allmon, 1996; Smith, 2011); for the above reasons a robust phylogeny for busyconine whelks is currently not available. Ecometric techniques are therefore ideal for investigating the history of anti-predatory defenses within *Busycon*, as they combine trait expression and ecology independent of a rigorous phylogenetic context (Eronen et al., 2010; Polly et al., 2011).

Pliocene-Pleistocene Environmental setting

The Western Atlantic during the Pliocene was characterized by an abundant and diverse molluscan fauna (Allmon et al., 1993; 1996a) and high levels of biological productivity (Allmon et al., 1996b; Allmon, 2001). Regionally, in Florida, the early Pliocene supported high marine vertebrate diversity, which declined in the late Pliocene (Emslie & Morgan, 1994; Morgan, 1994). Stable isotopic evidence derived from gastropods and bivalves suggest that seasonal upwelling was common, leading to increased productivity and nutrient availability (Jones & Allmon, 1995). Both invertebrate and vertebrate fossils from Florida in the early to middle Pliocene indicate high levels of marine productivity, with high seabird abundance, turritellid gastropod beds, and a diverse marine mammal fauna (see Allmon et al., 1996b and Allmon, 2001 for a review of invertebrate and vertebrate evidence). Molluscan predators were abundant and competitive, and possibly more powerful than Pleistocene faunas (Dietl et al., 2004).

Faunal evidence suggests a dramatic decline in paleo-productivity and temperature in the late Pliocene and Pleistocene in the tropical western Atlantic and southeastern United States (e.g., Allmon, 1992; Allmon et al., 1996; Allmon, 2001; Todd et al., 2002) associated with the

closing of the Central American Seaway (CAS) approximately 3 million years ago. The closure of the CAS dramatically changed circulation patterns in the Western Atlantic Ocean (see Allmon, 2001 for a review). This event is linked to high turnover rates within molluscan fauna (e.g., Stanley & Campbell, 1981; Stanley, 1986; Jackson et al., 1993; Todd et al., 2002), with bivalve extinction and origination rates of 47.9% and 26.7%, respectively (Stanley, 1986) and gastropod extinction and origination rates of 62.4% and 55.2% (Allmon et al., 1996a). Predatory gastropods were hard hit in terms of abundance, though diversity patterns and declines were mixed (Todd et al., 2002); there is some evidence that heavily sculptured gastropod genera experienced higher extinction rates than more weakly sculptured genera (Vermeij & Petuch, 1986), that more generally morphologically variable taxa were less vulnerable to extinction (Kolbe et al., 2011), and that filter feeding organisms had increased extinction vulnerability (e.g., Allmon, 1992; Todd et al., 2002). The remaining fauna likely experienced a less risky post-extinction biotic environment (Dietl et al., 2004). In Florida, diversity from the Pliocene to the Recent has not noticeably declined; diversity further north in the Carolinas and Virginia has been seriously diminished in the same time interval (Allmon et al., 1993, 1996a; Dietl et al., 2004).

Several specific predictions can be made pertaining to morphological changes within *Busycon* and *Sinistrofulgur*, given this environmental setting during the Pliocene and Pleistocene:

1. The tumid ridge and large spines should be most abundant during the middle Pliocene, and body size and shell thickness should be highest during this interval.
2. Selection for the maintenance of anti-predator adaptations (as indicated by percent individuals possessing repair scars) is also expected to be highest during the middle Pliocene.

3. Expression of the tumid ridge and large spines as well as shell thickness and maximum length should decrease in the early and middle Pleistocene, and repair frequency should also decline.

Additionally, I investigated whether the expression of morphological adaptations differed between dextral and sinistral whelks. As sinistrality confers anti-predatory benefits (Dietl & Hendricks, 2006), sinistral whelks are predicted to have lower development of costly adaptations where they co-exist with dextral whelks.

METHODS

Specimens & Stratigraphy

Over 300 lots of specimens of *Busycon* and *Sinistrofulgur,* totaling approximately 800 individuals from the Paleontological Research Institution and from private collections were examined and scored for several features (shell length from apex to canal, thickness at the shoulder and canal, degree of development of the tumid ridge, and degree of development of spines). Specimens were classified by stratigraphic unit and by state, and grouped into six time bins based on equivalency of stratigraphic units (based on Cronin et al., 1984 & Kolbe et al., 2011). Samples spanned the mid Pliocene to Recent temporally, with spatial coverage extending in the Pliocene from Virginia to Florida, and Pleistocene coverage shifting slightly south to include North Carolina and Florida predominantly, with Recent spatial coverage limited to *Busycon* extending from Virginia to Florida (Table 4.1).

Stratigraphic Unit/Locality	Time Bin/Approximate Age
Recent	(6) Recent
6.1: *Cape Charles, Virginia* (14)	
6.2: *Beaufort, North Carolina* (71)	
6.3: *Charleston, South Carolina* (25)	
6.4: *Wassaw Sound, Georgia* (44)	
6.5: *St. Catherine's Island, Georgia* (32)	
6.6: *Sapelo Island, Georgia* (34)	
6.7: *St. Simon's Island, Georgia* (22)	
6.8: *Amelia Island, Florida* (15)	
6.9: *Cape Canaveral, Florida* (10)	
Canepatch Formation	(5) Late Pleistocene (~80,000 KA [York et al., 2001])
5.1: *Edisto Pit, South Carolina* (n=34)	
Ft. Thompson Formation	(4) Middle-Late Pleistocene (~150,000-950,000 KA)
4.1: *Leisey Pit, Florida* (n=58)	
4.2: *Quality Materials, Florida* (n=80)	
4.3: *Philman Pit, Florida* (n=41)	
Bermont Formation	(3) Early-Middle Pleistocene (~1-1.65 MYA)
3.1: *Bermont Road Pit, Florida* (n=16)	
3.2: *Longan Lakes, Florida* (n=40)	
3.3: *GKK* (n=20)	
Waccamaw Formation (Lower)	(2) Early Pleistocene (~1.65-2.6 MYA)
2.1: *Old Dock, North Carolina* (n=3)	
2.2: *Acme, North Carolina* (n=12)	
2.3: *Resigter Quarry, North Carolina* (n=62)	
Caloosahatchee Formation	
2.4: *Cochran's Pit, La Belle, Florida* (n=7)	
2.5: *La Belle Picnic Grounds, Florida* (n=23)	
2.6: *Okeelanta, Florida* (n=19)	
2.7: *C2-C2, La Belle, Florida* (n=15)	
James City Formation (Lower)	
2.8: *Lee Creek Mine, North Carolina* (n=54)	
Nashua Formation	
2.9: *Dickerson Pit, Florida* (n=83)	
Duplin Formation	(1) Upper Pliocene (~2.6-3.6 MYA)
1.1: *Natural Well, North Carolina* (n=13)	
1.2: *Holmes Pit, North Carolina* (n=3)	
1.3: *Tar Heel, North Carolina* (n=43)	
1.4: *Kenansville, North Carolina* (n=15)	
1.7: *Strickland's Marl Pit, North Carolina* (n=5)	
Pinecrest Formation (Upper and Lower)	
1.5: *Sarasota, Florida* (n=16)	
Jackson Bluff Formation, Alum Bluff	
1.8: *Liberty County, Florida* (n=8)	
Yorktown Formation (Undifferentiated)	
1.6: *York River, Yorktown, Virginia* (n=47)	

Table 4.1. Stratigraphic units, localities, sample sizes and time bins.

Morphometrics

Shell length from the tip of the siphonal canal to the apex was measured to the nearest tenth of a millimeter on each specimen (Figure 4.1). Average length for all specimens over 100 mm in length, and the average length of the largest five specimens were calculated for each state and time bin. Length was also used to bin samples for further analyses: a 100-200 mm long size bin was established and used for additional linear morphometrics. This size bin was chosen to reflect when adaptations become apparent ontogenetically within *Busycon* and *Sinistrofulgur*, as juveniles (at least within *Busycon*) may not express adaptations as strongly as mature individuals (Appendix 4.1). The upper limit on size (200 mm) was chosen to reflect the fact that many samples do not have individuals above 200 mm (Figures 4.5, 4.6), which could result from a variety of factors, including differences in longevity or changes in growth patterns through time. For a study of adaptation through time, it is necessary to exclude juveniles, which may not have developed adaptations, and very old individuals, which may experience different growth patterns, to prevent shifts in ontogenetic age from sample to sample from skewing results.

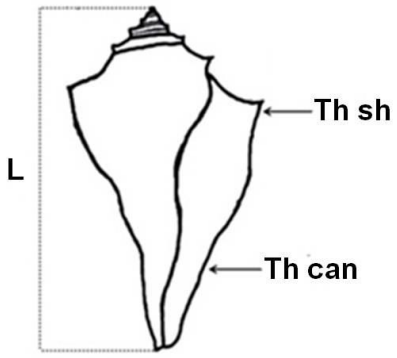

Figure 4.1. Linear morphometrics. L indicates shell length; Th sh indicates where shell thickness at the shoulder was measured; Th can indicates where shell thickness at the canal was measured.

Shell thickness was measured both at the shoulder and halfway down the siphonal canal, to the nearest tenth of a millimeter (Figure 4.1., th sh and th can). Thickness at the canal and at the shoulder for an individual of 150 mm shell length was predicted by fitting a linear regression of length vs. shell thickness to individuals between 100-200 mm from each state within each time bin, and then calculating shell thickness. Spines were divided into 4 categories (Figure 4.2.) and scored from least developed (0) to most developed (3) by labeling shells as possessing either spines (scored as 3, large protrusions from the shoulder), tubercules (scored as 1, low bumps protruding slightly from the shoulder), a mix of spines and tubercules (scored as 2), or no shoulder ornamentation (scored as 0). The tumid ridge was classified into two categories (Figure 4.3.): "present and prominent to present and moderately developed" (scored as 1), or "extremely weakly developed or absent" (scored as 0). Differences in the development of the ridge and spines between time periods, states, and chiralities were also assessed using a Mann-Whitney U test (or Kruskal-Wallis, for larger comparisons) in PAST (Hammer et al., 2001).

Figure 4.2. Differential development of spines. Shell on far left has no shoulder ornamentation (scored as zero); center shell has low tubercules along the shoulder (scored as 1), and far right shell has spines (scored a 3). Shells with a mix of tubercules and spines would score as 2. Scale bars represent 10 mm.

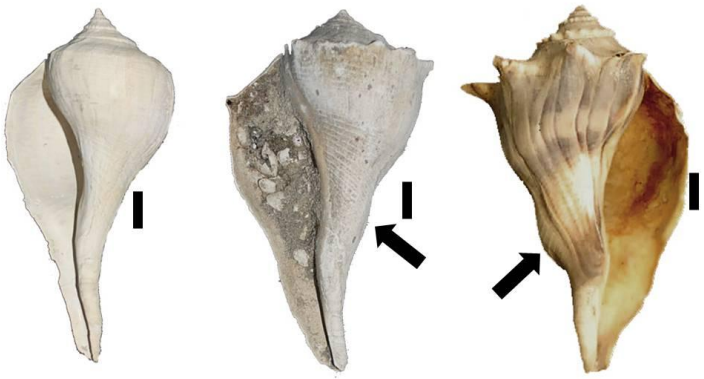

Figure 4.3. Differential development of tumid ridge. Shell on far left has no ridge (scored as zero); center shell has slight ridge indicated by arrow (scored as 1), and far right shell has prominent ridge indicated by arrow (also scored as 1). Scale bars represent 10 mm.

Repair Scar Analysis

I counted repair scars, which are conspicuous marks occurring when an animal is attacked and severely damaged but survives to repair it shell, on the final whorl of each specimen (Figure 4.4). Only severe repair scars (scars that: 1.) cut across multiple growth intervals; or, 2.) represented jagged breaks extending over most of the length (>75%) of the apertural lip) were counted. Repair frequency was calculated as the percentage of individuals possessing at least one severe repair scar (percentage method, Alexander & Dietl, 2003). As morphology affects the accumulation of repair scars, I initially compared repair frequency across all times bins, and then limited comparisons of repair frequencies to those specimens either lacking a tumid ridge or only possessing a very weakly developed tumid ridge (scored as 0 or 1). Specimens were grouped into a 100-200mm size bin, and individuals above or below this size were excluded from the analysis. Size is known to affect predation risk and the likelihood of accumulating repair scars (e.g. Paine, 1976; Elner & Hughes, 1978; Vermeij, 1982; Boulding, 1984; Creswell & McLay, 1990; Juanes & Hartwick, 1990; West et al., 1991; Yamada & Boulding, 1998; Leighton, 2002), and while this grouping does not break size down as finely as it could be

broken down, combining size classes at some level was necessary to maintain sample sizes that accurately reflected biotic signals.

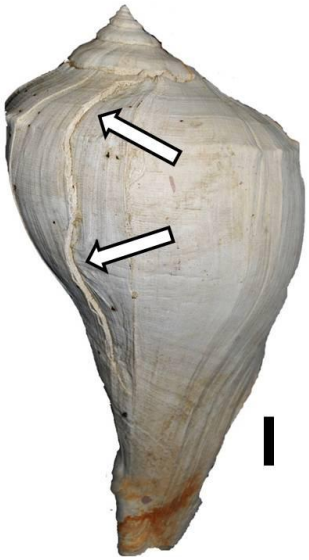

Figure 4.4. Repaired shell. Note jagged break extending from shoulder to canal, indicated by arrows. Scale bar=20 mm.

RESULTS

Morphometrics: Dextral Whelks

Dextral whelks show both spatial and temporal variation in the expression of adaptive traits. Average length and maximum length peak in the Pleistocene, whereas thickness at the canal is predicted to be highest for similarly sized individuals in the Pliocene (Table 4.2). Thickness at the canal drops across the Plio-Pleistocene boundary. Thickness at the shoulder does not change substantially across the Plio-Pleistocene boundary (Table 4.2), and does not seem to show specific trends through time. Expression of the tumid ridge is highest in the Pliocene, and decreases greatly in the Early Pleistocene. The tumid ridge is not expressed again in great frequencies until the Recent, where it is most abundant in Georgia and Florida, in

contrast to its' prior highest abundance in the northernmost part of *Busycon*'s range (Table 4.2). Spines also show variable expression through time, with high proportions of Pliocene populations expressing spines, low proportions of early to middle Pleistocene populations expressing spines, and then very strong expression of spines for all individuals throughout the geographic range in the late Pleistocene and Recent (Figure 4.5, Appendix 4.2).

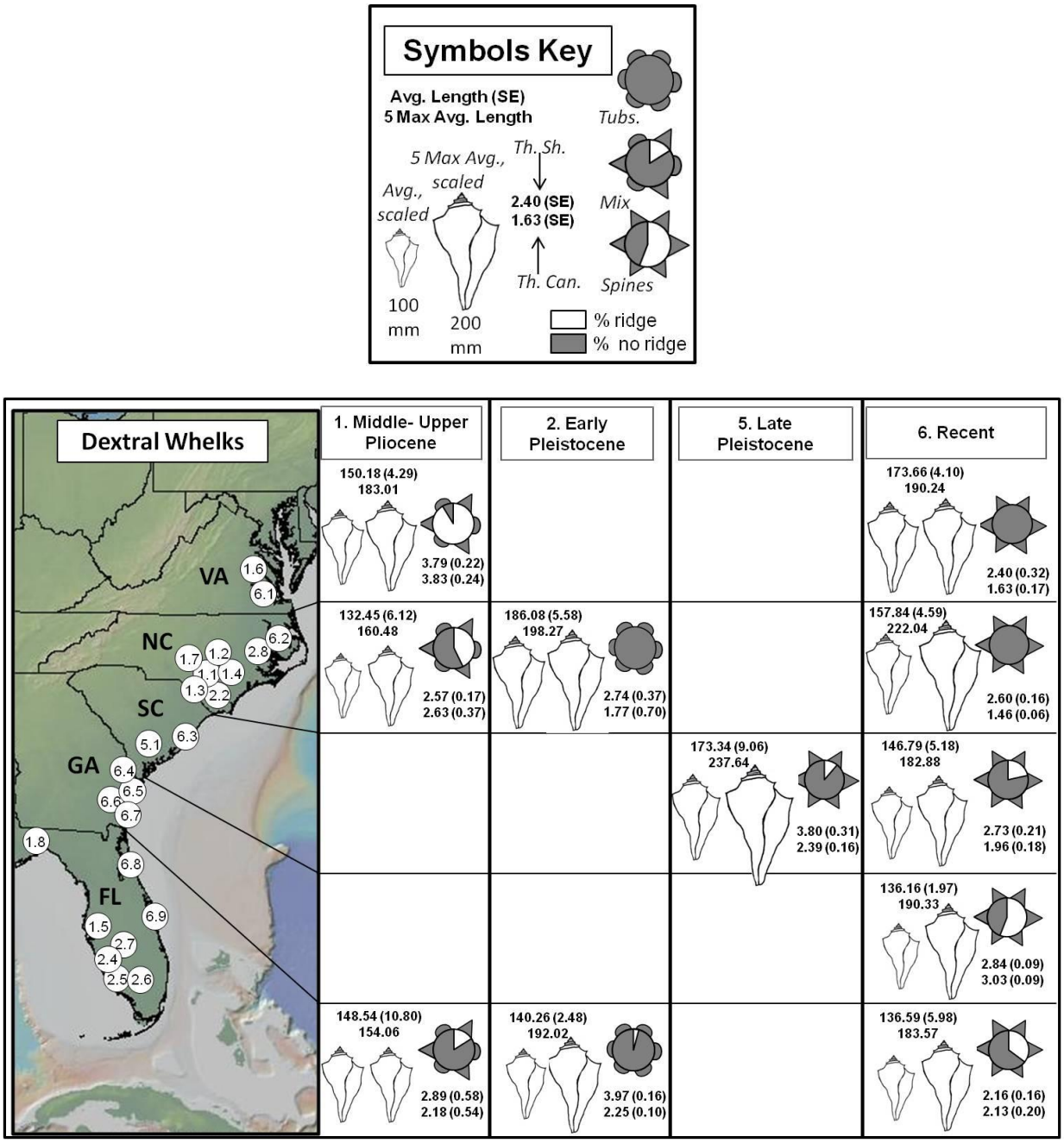

Figure 4.5. Trait values over time, all specimens between 100-200mm, dextral whelks. Mean length for all specimens and mean length for five largest specimens given numerically; pictures are scaled as indicated in key. Shell thickness at the shoulder and ridge are given as predicted values at a length of 150 mm, SE for population given in parentheses. Map from http://www.geomapapp.org/.

Spatially, average and maximum lengths generally tend to be highest to the north, and thickness at the canal is highest to the north. In the Recent, thickness at the canal is highest in the southern part of the modern species' range. The percentage of individuals with a ridge is high in Virginia in the Pliocene, and low in all states during the Pleistocene. The expression of spines through time is not significantly different between states.

Morphometrics: Sinistral Whelks

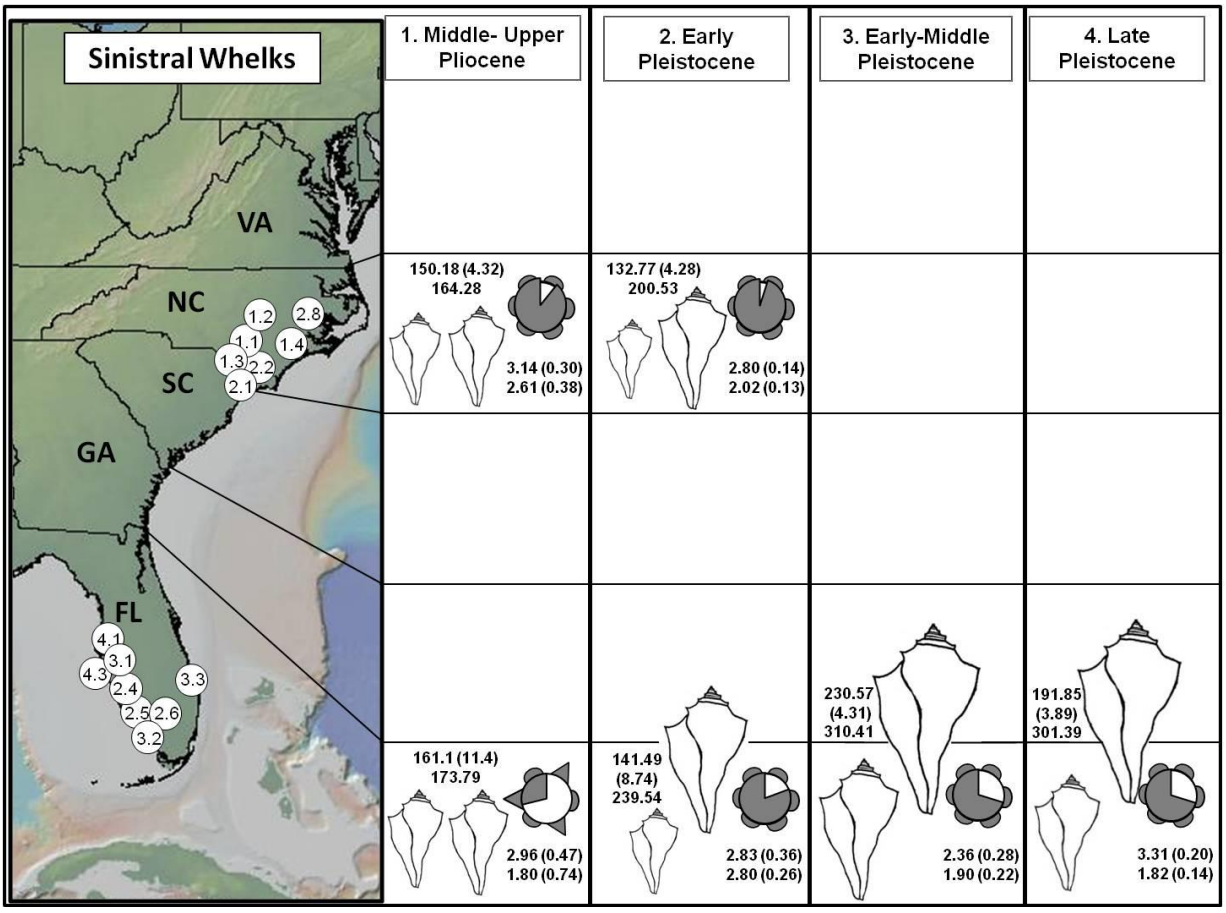

Figure 4.6. Trait values through time, sinistral whelks. Mean length for all specimens and mean length for five largest specimens given numerically; pictures are scaled as indicated in key for Figure 4.5. Shell thickness at the shoulder and ridge are given as predicted values at a length of 150 mm, SE for population given in parentheses. Map from http://www.geomapapp.org/.

For sinistral whelks, average length increases from the Pliocene to Middle-Late Pleistocene for sinistral whelks, as does maximum length. Predicted thickness at the canal shows no clear trend through time in sinistral whelks (Figure 4.6, Appendix 4.3), and predicted

thickness at the shoulder also does not seem to vary systematically through time. The proportion of individuals possessing a tumid ridge declines non-significantly across the Plio-Pleistocene boundary, and increases slightly in the Middle to Late Pleistocene. Expression of spines shows a similar pattern, decreasing significantly from the Pliocene into the Early Pleistocene, and then increasing slightly in the Middle to Late Pleistocene.

Body size is higher in Florida in the Pliocene and Pleistocene for sinistral whelks, though predicted shell thickness peaks in North Carolina. The percentage of individuals possessing a tumid ridge is significantly higher in Florida as compared to North Carolina in both the Pliocene and Pleistocene. Spines do not seem to vary systematically spatially.

Morphometrics: Comparisons between chiralities

Sinistral whelks tend to be larger in the Pliocene and early Pleistocene than coeval dextral whelks within states. Thickness at the canal and shoulder do not seem to show strong trends either spatially or through time between chiralities, though dextral whelks are predicted to be slightly thicker at the shoulder in both Florida and North Carolina during the Pliocene, and sinistral whelks in both Florida and North Carolina are predicted to be thicker at the canal during the Pliocene.

Chiralities show a marked and statistically significant ($p<0.05$, Mann-Whitney U) difference in where the ridge is most abundant in the Pliocene; whereas at this time, sinistral whelks show the highest proportion of individuals with a ridge in Florida, dextral whelks with ridges are most abundant to the north, in Virginia. In the early Pleistocene, development of the tumid ridge is significantly higher for sinistral whelks in Florida ($p<0.01$, Mann-Whitney U); differences between chiralities in North Carolina are not significant. Small sample sizes for the North Carolina population of dextral whelks may have limited statistical power. Spines are best

developed in the Pliocene for dextral whelks; in the early Pleistocene, differences are less pronounced. Differences in spinosity between chiralities are not significant.

Repair Scar Analysis

Individuals possessing a tumid ridge were excluded from repair scar analyses, as the tumid ridge affects the likelihood of incurring severe damage during predatory attacks and thus the likelihood of accumulating repairs (Chapter 3). Excluding individuals with this adaptation ensured that repair frequencies were not controlled by populations with higher or lower than average frequencies of individuals possessing a tumid ridge. Within chiralities, repair frequencies do not seem to change directionally through time, though spatial variation is considerable. For instance, in the Recent, repair frequency in dextral whelks varies from 0.12 to 0.75; in the early Pleistocene values for sinistral whelks vary from 0.10 to 0.32. In the Pliocene, repair scar frequencies were slightly lower in sinistral whelks as compared to dextral whelks, as noted in a prior study (Figure 4.7., Appendix 4.4, Dietl & Hendricks, 2006). Pleistocene populations do not show this pattern.

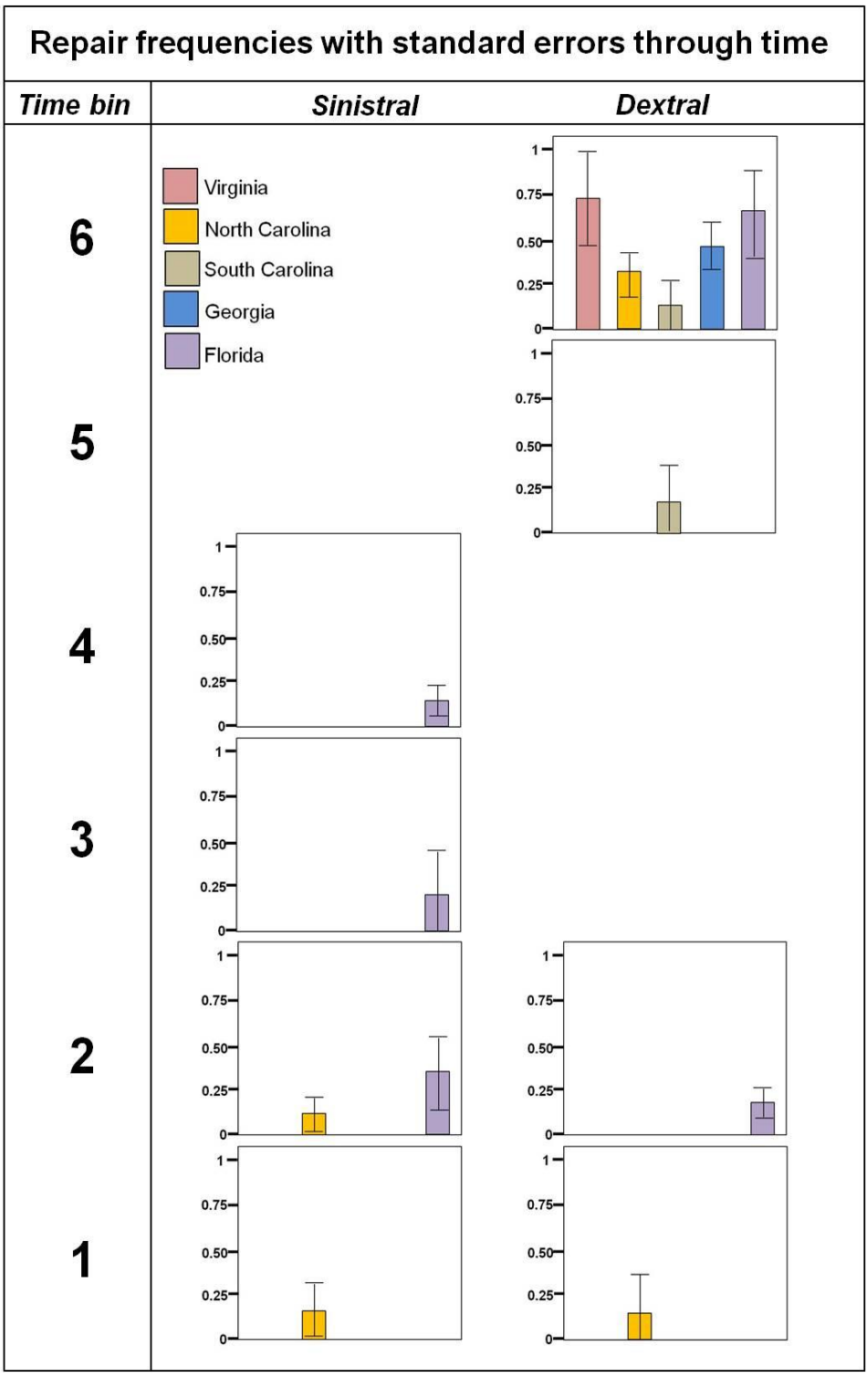

Figure 4.7. Proportion of population with at least one severe repair scar on final whorl through time, all specimens between 100-200 mm, excluding specimens with tumid ridges. Samples with less than 10 individuals were excluded.

DISCUSSION

Ecometric predictions linking the spatial and temporal distribution of costly adaptive traits and known environmental conditions are well-matched from the Pliocene to the Recent. Costly morphological adaptations are generally predicted to be most likely to occur in times elevated predation risk (e.g., Vermeij, 2002, 2012), and this holds in busyconine whelks. The tumid ridge, large spines, and increased shell thickness function well as ecometric indicators of competitive and risky environments. The maintenance of well-ornamented shells in the Pliocene correlates with more permissive (in terms of productivity and temperature) and, as a consequence, risky, escalated evolutionary environments (e.g., Stanley & Campbell, 1981; Jackson et al., 1993; Jones & Allmon, 1995; Allmon et al., 1996; Todd et al., 2002).

Subsequent loss of or reduced development of adaptive features (e.g., the tumid ridge and spines) is interpreted as s response to lowered predation risk resulting from extensive extinctions and faunal turnover caused by the closure of the Central American Seaway (e.g., Stanley, 1986; Vermeij, 1989; Allmon, 1992; Jackson et al., 1993; Dietl et al., 2004).

The maintenance of well-developed tumid ridges and large spines in modern whelks (species *B. carica*) suggests a continued ecologic recovery from the Plio-Pleistocene event. Development of these features is restricted to populations south of Cape Hatteras, in the warm-temperate to sub tropical Carolinian Province (Chapter 3); in general, this region is predicted to experience higher predation pressure than cooler areas further north. Repair scar data and predator abundance also peak to the South, supporting the utility of these traits as ecometric indicators. Thus, the modern distribution of morphologies supports the hypothesis that the

development of these morphological adaptations are only likely to occur in more escalated environments, such as those that existed in the Pliocene.

Chiralities show different patterns in where adaptations are most prevalent through time and across localities. Dextral whelks are predicted to be slightly thicker at the shoulder in the Pliocene and early Pleistocene, and also are slightly more spinose. The reduced expression of these adaptations in sinistral whelks may reflect lowered predation pressure for this chirality. As sinistral whelks are more difficult for dextral predators to handle (e.g., Inoda et al., 2003; Dietl & Hendricks, 2006; Hoso et al., 2007), less frequent and less successful attacks on sinistral whelks should have diminished selection for the maintenance of costly adaptations.

Ecometrics explicitly ignores taxonomy, using a taxon-free approach to examine changes in morphology through time. In this case, morphologically variable short-lived lineages (e.g., *Busycon rucksorum*; *Busycon gilbertii*) that may be less likely to express adaptations may modify patterns between localities and through time. While sinistral whelks do not appear to have this problem, as there are less offshoots to the lineage, future work should investigate the magnitude and possible directionality of variation in a wide range of dextral species and sub-species, as well as differences in spatial distributions through time.

Trends in repair frequencies were generally not significant through time. Repair scar data (and therefore estimates of relative predation pressure) is substantially affected by differences in growth rate, which in the modern varies both with latitude and with morphology (Chapter 2; Chapter 3). At this time, geochemical analysis of growth rates for busyconine whelks have not been performed beyond Recent species. Future work should examine the evolution of growth rates over the geologic time span of this genera. It is likely that growth rates dropped across the Plio-Pleistocene boundary: less productive conditions are generally theorized to dampen growth

rates, as are cooler conditions. There is some evidence that this extinction selectively extirpated larger, more rapidly growing species (e.g., Petsios & Allmon, 2012); if this pattern holds within whelks, higher repair frequencies might shift definitively to the Pliocene. Until growth rate studies are undertaken, however, the magnitude of this problem cannot be ascertained.

CONCLUSIONS

Traits such as the tumid ridge and large spines are effective ecometric indicators of both enhanced predation, and enhanced environmental permissivity. The temporal distribution of these adaptations mirrors knowledge of when environmental conditions were most risky (in the Pliocene), and reflect deteriorating abiotic environments and consequently diminished biotic risks in the Pleistocene. Ecometrics, in the case of busyconine whelks, provides additional information on both the effects of the Plio-Pleistocene boundary event on ecological conditions, and on the ongoing faunal recovery. Current trait distribution mirrors maximum predator abundance and selection for the development of adaptations, though corrections for growth rate are necessary for the Recent pattern to become apparent (Chapter 1, Chapter 2, Chapter 3). Future work in this system should focus on constraining growth rates through time, as different growth rates can modify apparent repair frequency (Chapter 1, Chapter 3). Additional work should also examine the modern distribution of adaptations in sinistral whelks, which is spatially offset from where adaptations are most abundant in dextral whelks, in the context of predation intensity.

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APPENDIX

Appendix 4.1. Body size and adaptation

To explore differences in the development of adaptations between body sizes and to determine an appropriate size range for further analyses, I used simple linear morphometrics to compare individuals from 0-100 mm in length and from 100-200 mm in length in two populations (Table 4.A.1):

1. A Pliocene population from North Carolina, and
2. An early Pleistocene population from Florida

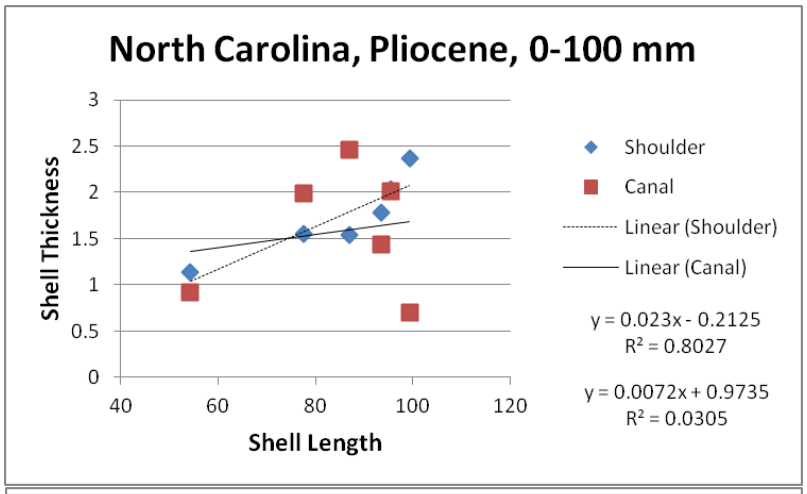
Predicted thickness at the shoulder and at the siphonal canal for a 100 mm shell length individual was calculated by fitting linear regressions to individuals within each size class in the respective populations (Figure 4.A.1). Additionally, the proportion of individuals possessing a tumid ridge and the degree of development of spines were quantified, using the above basic methods. The two populations (North Carolina vs. Florida) span the extinction boundary, so degree of development of adaptations is expected to be variable between the populations; these methods allowed assessment of whether adaptations differed between the two size classes.

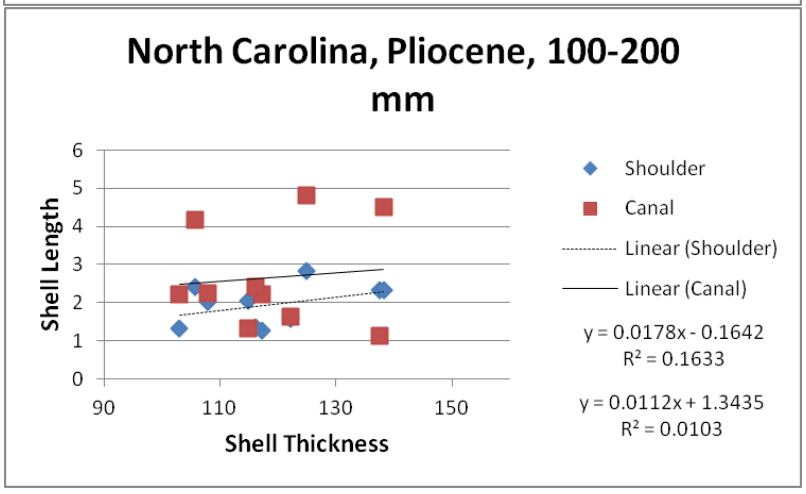
Population, size class, and N	**Predicted thickness at shoulder**	**Predicted thickness at canal**	**Proportion of individuals with ridge**	**Spinosity**
Pliocene, North Carolina, 0-100 mm (N=6)	2.09	1.69	0.20	0.80
Pliocene, North Carolina, 100-200 mm (N=10)	2.46	1.62	0.44	2.13
Pleistocene, Florida, 0-100 mm (N=10)	2.02	1.25	0.00	0.07
Pleistocene, Florida, 100-200 mm (N=69)	2.06	1.33	0.00	0.59

Table 4.A.1. Comparisons of the relative development of adaptations between size classes.

The Pliocene population showed differential development of both spines and the tumid ridge between size classes, with larger individuals expressing these adaptations much more

strongly. Predicted thickness at the shoulder and canal also varied, with thickness at the shoulder varying by almost half a millimeter. The Pleistocene population did not express a tumid ridge (as expected after the extinction boundary); however, expression of spines varied between size classes, with the larger size class having on average better developed shoulder ornamentation. Predicted thickness at the shoulder and ridge also varied slightly, with higher values predicted for the larger size class.





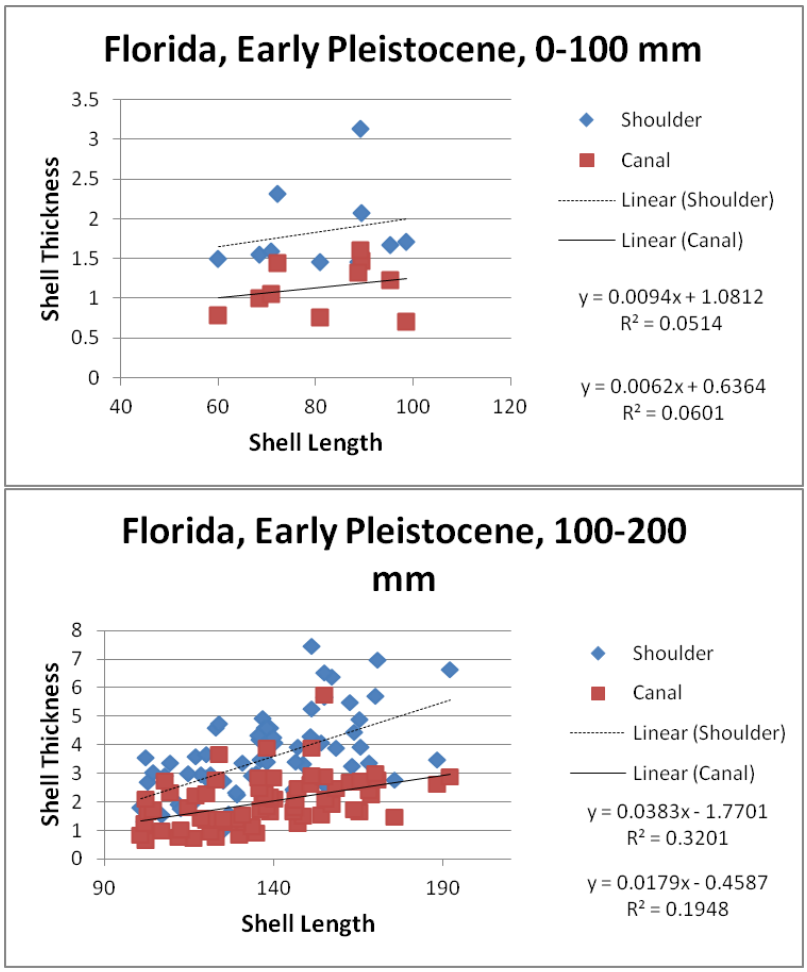


Figure 4.A.1. Linear regressions of shell length vs. measured thickness at the shoulder and canal. Equations are listed (from top to bottom) as derived from the shoulder, and then as derived from the canal measurements.

APPENDIX

Appendix 4.2. Trait values through time, dextral whelks.

Time Interval, State	Avg. Length	Avg. Length, 5 max	Pred. Th (150 mm), Canal	Pred. Th (150 mm), Shoulder	% ridge	Spines	N
6. Recent							
Florida	136.59	183.57	2.13	2.16	0.38*^	3.00^	24
Georgia	136.16	190.33	3.03	2.84	0.58*	3.00	131
South Carolina	146.79	182.88	1.96	2.73	0.24*	3.00	25
North Carolina	157.84	222.04	1.46	2.60	0.00*^	3.00^	55
Virginia	173.66	190.24	1.63	2.40	0.00*	3.00	14
5. Late Pleistocene							
South Carolina	173.34	237.64	2.39	3.80	0.12	2.88	17
2.Early Pleistocene							
Florida	140.26	192.02	2.25	3.97	0.01^	0.59^	92
North Carolina	186.08	198.27	1.77[1]	2.74	0.00	0.86^	7
1.Pliocene							
Florida	148.56	154.06	2.18	2.89	0.14	1.86^	8
North Carolina	132.45	160.48	2.63	2.57	0.44^	2.06^	19
Virginia	150.18	183.01	3.83	3.79	0.92	2.17	39

Appendix 4.2. Trait values over time, all specimens above 100 mm, dextral whelks. *indicates values that were significantly different ($p<0.05$) between states within time bins, ^ indicates values that were significantly different across time bins for specific states. ~indicates that statistic tests were not performed due to small sample sizes. [1.] Calculations for thickness at the shoulder and ridge included individuals between 50-250 mm in length, as sample size was otherwise too small.

APPENDIX

Appendix 4.3. Trait values through time, sinistral whelks.

Time Interval, State	Avg. Length	Avg. Length, 5 max	Pred. Th (150 mm), Canal	Pred. Th (150 mm), Shoulder	% ridge	Spines	N
4. Middle-Late Pleistocene							
Florida	191.85	301.39	1.82	3.31	0.28	1.38^	93
3. Middle Pleistocene							
Florida	230.57	310.41	1.90	2.36	0.29	0.93	14
2. Early Pleistocene							
Florida	141.49	239.54	1.80	2.83	0.23*	0.62^	26
North Carolina	132.77	200.53	2.02	2.80	0.02*	0.74^	50
1. Pliocene							
Florida	161.10	173.79	1.80	2.96	0.71*	1.75^	8
North Carolina	150.18	164.28	2.61	3.14	0.07*	1.33^	32

Appendix 4.3. Trait values through time, all specimens over 100 mm, sinistral whelks. *indicates values that were significantly different ($p<0.05$) between states within time bins, ^ indicates values that were significantly different across time bins for specific states. ~indicates that statistic tests were not performed due to small sample sizes.

APPENDIX

Appendix 4.4. Repair frequency through time.

Time Interval, State	Sinistral RF	N	Dextral RF	N
6. Recent				
Florida	-	-	0.67	15**
Georgia	-	-	0.48	55
North Carolina	-	-	0.33	55
South Carolina	-	-	0.12	19**
Virginia	-	-	0.75	14**
5. Late Pleistocene				
South Carolina	-	-	0.36	15**
4. Middle-Late Pleistocene				
Florida	0.13	67	-	-
3. Middle Pleistocene				
Florida	0.20	10**	-	-
2. Early Pleistocene				
Florida	0.32	20	0.17	90
North Carolina	0.1	48	-	-
1.Pliocene				
Florida	-	-	-	-
North Carolina	0.14	27	0.33	10**
Virginia	-	-	-	-

Appendix 4.4. Repair frequencies through time for sinistral and dextral whelks, with sample sizes.

REFERENCES

Abbott, R. T. 1974. American Seashells. 2nd Edition. Van Nostrand Reinhold Co., New York. 663 p.

Alexander, R. R. and G. P. Dietl. 2003. The fossil record of shell-breaking predation on marine bivalves and gastropods. Pp. 141–176 *in* P. H. Kelley, M. Kowalewski, and T. A. Hansen, eds. Predator-prey interactions in the fossil record. Kluwer Academic/Plenum, New York.

Allmon, W.D. 1992. Role of temperature and nutrients in extinction of turritelline gastropods: Cenozoic of the northwestern Atlantic and northeastern Pacific. Palaeogeography, Palaeoclimatology, Palaeoecology 92:41-54.

Allmon, W.D. 1996. Evolution and systematic of Cenozoic American Turritellidae (Gastropoda) I. Paleocene and Eocene species related to "Turritella mortoni Conrad" and "Turritella humerosa Conrad" from the U.S. Gulf and Atlantic Coastal Plains. Palaeontographica Ameicana 59:1-134.

Allmon, W.D. 2001. Nutrients, temperature, disturbance, and evolution: a model for the late Cenozoic marine record of the western Atlantic, Palaeogeography, Palaeoclimatology, Palaeoecology, Volume 166, Issues 1–2, : 9-26.

Allmon,W.D., G. Rosenberg, R.W. Portell, K. Schindler. 1993. Diversity of Pliocene to Recent Atlantic coastal plain mollusks. Science, 260 (1993), pp. 1626–1628

Allmon, W.D., G. Rosenberg, R.W. Portell, K. Schindler. 1996a. Diversity of Pliocene–Recent mollusks in the western Atlantic: extinction, origination, and environmental change. J.B.C. Jackson, A.F. Budd, A.G. Coates (Eds.), Evolution and Environment in Tropical America, University of Chicago Press, Chicago, IL.

Allmon, W.D., S.D. Emslie, D.S. Jones, G.S. Morgan. 1996b. Late Neogene oceanographic change along Florida's west coast: evidence and mechanisms. Journal of Geology, 104 (1996), pp. 143–162

Bailey, I.W. & E.W. Sinnott, 1915. A botanical index of Cretaceous and Tertiary climates. Science 41(1066):831-834.

Bailey, I.W. & E.W. Sinnott, 1916. The climatic distribution of certain types of angiosperm leaves. American Journal of Botany 3(1):24-39.

Berlocher, S. H. 2000. Allozyme variation in *Busycon* Whelks (Gastropoda: Melongenidae). Biochemical Genetics38: 285-295.

Boulding, E.G. 1984. Crab-resistant features of shells of burrowing bivalves: decreasing vulnerability by increasing handling time. J. Exp. Mar. Biol. Ecol., 76: 201-223.

Brookes J.I., & R. Rochette. 2007. Predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*: developmental by-product or active physiological response? Journal of Evolutionary Biology 20: 1015-1027.

Creswell, P.D. & C. L. McLay. 1990. Handling times, prey size and species selection by *Cancer novaezelandiae* (Jacquinot, 1853) feeding on molluscan prey. J. Exp. Mar. Biol. Ecol. 140:13-28.

Cronin, T.M., Bybell, L.M., Poore, R.Z., Blackwelder, B.W., Liddicoat, J.C. & J.E. Hazel. 1984. Age and correlation of emerged Pliocene and Pleistocene deposits, U.S. Atlantic Coastal Plain. Palaeogeography, Palaeoclimatology, Palaeoecology 47:21-51.

Delgado, G. A., Glazer, R. A., & N.J. Stewart. 2002. Predator-Induced Behavioral and Morphological Plasticity in the Tropical Marine Gastropod *Strombus gigas*. Biological Bulletins 203:112-120.

DeWitt, T.J., Robinson B.W., *& D.S.* Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive tradeoff for shell morphology. Evol. Ecol. Res. 2*:* 129–148.

Dietl, G.P. , Herbert, G.S. & G.J. Vermeij.2004. Reduced competition and altered feeding behavior among marine snails after a mass extinction. Science 306:2229-2231.

Dietl, G. P. & J.R. Hendricks. 2006. Crab scars reveal survival advantage of left-handed snails. Biology Letters 2:439-442.

Edwards, A. L. 1988. Latitudinal clines in shell morphologies of *Busycon carica* (Gmelin 1791). Journal of Shellfish Research 7(3):461-466.

Edwards, A. L. & M.G. Harasewych. 1988. Biology of the Recent Species of the Subfamily Busyconinae. Journal of Shellfish Research 7:467-472.

Edwards, A. L. & C.M. Humphrey. 1981. An electrophoretic and morphological survey of *Busycon* occurring in Wassau Sound, Georgia. Nautilus 95:144

Elner, R. W., & R.N. Hughes. 1978. Energy maximization in the diet of the shore crab, *Carcinus maenas*. Journal of Animal Ecology, 47(1): 103-116.

Emslie, S.D. & G.S. Morgan. 1994. A catastrophic death assemblage and paleoclimatic implications of Pliocene seabirds of Florida. Science 264:684–685.

Eronen, J.T., Polly, P.D., Fred, M., Damuth, J., Frank, D.C., Mosbrugger, V., Scheidegger, C., Stenseth, N.C. & M. Fortelius. 2010. Ecometrics: the traits that bind the past and present together. Integrative Zoology 5(2):88-101.

Geller, J.B. 1990. Consequences of a morphological defense: growth, repair and reproduction by thin-and thick-shelled morphs of *Nucella emarginata* (Deshayes)(Gastropoda:Prosobranchia). J. Exp. Mar. Biol. Ecol. 144:173-184.

Hammer, Ø., Harper, D.A.T., & P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 9pp.

Harasewych, M.G. 1982. The evolution and zoogeography of the subfamily Busyconinae (Gastropoda: Melongenidae). University of Delaware, Ph.D. dissertation. 216 p.

Hollister, S. C. 1958. A Review of the Genus *Busycon* and its allies- Part I. Paleontographica Americana 4:49-126.

Hoso, M., Asami, T. & M. Hori. 2007. Right-handed snakes: convergent evolution of asymmetry for functional specialization. Biology Letters 3:169-172.

Inoda, T., Hirata, Y. & S. Kamimura. 2003. Asymmetric mandibles of water-scavenger larvae improve feeding effectiveness on right-handed snails. American Naturalist 162:811-814.

Jackson, J.B.C., Jung, P., Coates, A.G. & L.S. Collins. 1993. Diversity and extinction of tropical American mollusks and emergence of the Isthmus of Panama. Science 260(5114):1624-1626.

Jones, D. S. & W.D. Allmon. 1995. Records of upwelling, seasonality and growth in stable-isotope profiles of Pliocene mollusk shells from Florida. Lethaia 28: 61–74. doi: 10.1111/j.1502-3931.1995.tb01593.x

Juanes, F. & E.B. Hartwick. 1990. Prey size selection in Dungeness crabs: the effect of claw damage. Ecology 71: 744-758.

Kolbe, S.E., Lockwood, R., & G. Hunt. 2011. Does morphological variation buffer against extinction? A test using veneroid bivalves from the Plio-Pleistocene of Florida. Paleobiology 37(3):355-368.

Kosloski, M.E. & G.P. Dietl. 2011. The use of modern death assemblages to test the geographic mosaic theory of coevolution. Geological Society of America Abstracts with Programs, Vol. 43, No. 5, p. 504

Lawing, M.A., Head, J.J. & P. D. Polly. 2012. The Ecology of Morphology: The Ecometrics of Locomotion and Macroenvironment in North American Snakes. In Paleontology in Ecology and Conservation, Ed. J.Louys. Springer Berlin Heidelberg, pp.117-146.

Leidy, J. 1889. Remarks on the nature of organic species. Transactions of the Wagner Free Institute of Science, Philadelphia.

Leighton, L. R. 2002. Inferring predation intensity in the marine fossil record. Paleobiology, 28(3): 328-342.

Liu, L., Puolamäki, K., Eronen, J.T., Ataabadi, M.M., Hernesniemi, E., & M. Fortelius. 2011. Dental functional traits of mammals resolve productivity in terrestrial ecosystems past and present. Proc. R. Soc. B, published ahead of print March 28, 2012, doi:10.1098/rspb.2012.0211 1471-2954

Magalhaes, H. 1948. An ecological study of snails of the genus *Busycon* at Beaufort, North Carolina. Ecological Monographs 18, 377-409.

Morgan, G.S. 1994. Miocene and Pliocene marine mammal faunas from the Bone Valley Formation of central Florida. Proceedings of the San Diego Natural History Society 29:239–268.

Paine, R.T., 1962. Ecological diversification in sympatric gastropods of the genus *Busycon*. Evolution 16: 515–523.

Paine, R. T. 1976. Size-Limited Predation : An Observational and Experimental Approach with the *Mytilus- Pisaster* Interaction. Ecology, 57(5): 858-873.

Palmer, A.R. 1979. Fish predation and the evolution of gastropod shell sculpture: experimental and geographic evidence. Ecology 33:697-713.

Palmer, A.R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). Hydrobiologia 193 (1) 155-182.

Petsios, E. & W. D. Allmon. 2010. Phylogenetic analysis of the Florida Plio-Pleistocene *Turritella* using continuous characters. Geological Society of America *Abstracts with Programs*, Vol. 42, No. 5, p. 143.

Petuch, E.J. 1991. New gastropods from the Plio-Pleistocene of southwestern Florida and the Everglades basin. W.H. Dall Paleontological Research Center, Special Publication #1.

Petuch, E. J. 1994. Atlas of Florida Fossil Shells (Pliocene and Pleistocene Marine Gastropods) Chicago Spectrum Press, 394 pages

Polly, P.D., Eronen, J.T., Fred, M., Dietl, G.P., Mosbrugger, V., Scheidegger, C., Frank, D.C., Damuth, J., Stenseth, N.C., & M. Fortelius. 2011. History matters: ecometrics and integrative climate change biology. Proc. R. Soc. B 278(1709):1131-1140.

Royer, D.L. & P. Wilf. 2006. Why do toothed leaves correlate with cold climates? Gas exchange at leaf margins provides new insights into a classic paleotemperature proxy. Int. J. Plant Sci. 167(1):11-18.

Smith, U.E. 2011. Macroevolutionary patterns in the New Zealand Cenozoic turritellid genera Zeacolpus and Stiracolpus (Gastropoda: Turritellidae). Ph.D. Dissertation, Cornell University, Ithaca, NY.

Stanley, S.M. 1986. Anatomy of a regional mass extinction: Plio-Pleistocene decimation of the Western Atlantic bivalve fauna. Palaios 1(1):17-36.

Stanley, S.M. & L.D. Campbell. 1981. Neogene mass extinction of Western Atlantic mollusks. Nature 293:457-459.

Todd, J.A., Jackson, J.B.C., Johnson, K.G., Fortunato, H.M., Heitz, A., Alvarez, M. & P.J. Jung. 2002. The ecology of extinction: molluscan feeding and faunal turnover in the Caribbean Neogene. Proc. R. Soc. Lond. B 269:571-577.

Trussell, G.C. & R.J. Etter. 2001. Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. Genetica 112-113:321-337.

Trussell, G.C. & M.O. Nicklin. 2002. Cue sensitivity, inducible defense, and trade-offs in a marine snail. Ecology 83 (6), 1635-1647.

Vermeij, G. J. 1982. Unsuccessful Predation and Evolution. The American Naturalist 120 (6):701-720

Vermeij, G.J. 1987. *Evolution and Escalation.* An Ecological History of Life. Princeton University Press, Princeton, N. J. 527 pp.

Vermeij,G.J. 1989. Interoceanic differences in adaptation: effects of history and productivity. Marine Ecology Progress Series 57:293-305.

Vermeij, G. J. 1993. A Natural History of Shells. Princeton University Press, Princeton, New Jersey. 216 pp.

Vermeij, G.J. 2001. Innovation and evolution at the edge: origins and fates of gastropods with a labral tooth. Biological Journal of the Linnean Society 72:461-508.

Vermeij, G.J. 2002. The geography of evolutionary opportunity: hypothesis and two cases in gastropods. Integr. Comp. Biol. 42:935-940.

Vermeij, G.J. 2007. The ecology of invasion: acquisition and loss of the siphonal canal in gastropods. Paleobiology 33(3):469-493.

Vermeij, G.J. 2012. Crucibles of creativity: the geographical origins of tropical molluscan innovations. Evolutionary Ecology 26(2):357-373.

Vermeij, G.J. & E.J. Petuch. 1986. Differential extinction in tropical American mollusks. Endemism, architecture, and the Panama land bridge. Malacologia 27:29-41.

West, K., Cohen, A., & M. Baron. 1991. Morphology and behavior of crabs and gastropods from Lake Tanganyika, Africa: implications for lacustrine predator-prey coevolution. Evolution 45(3):589-607.

Wilf, P. 1997. When are leaves good thermometers? A new case for Leaf Margin Analysis. Paleobiology 23(3):373-390.

Wise, J., Harasewych, M.G., & R.T. Dillon Jr. 2004. Population divergence in the sinistral whelks of North America, with special reference to the east Florida ecotone. Marine Biology 145: 1167-1179.

Yamada, S.B. & E.G. Boulding. 1998. Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. J. Exp. Mar. Biol. Ecol. 220 (2): 191-211.

Zuschin, M., Stachowitsch, M., & R. J. Stanton Jr. 2003. Patterns and processes of shell fragmentation in modern and ancient marine environments. Earth Science Reviews 63: 33-82.